COMPARATIVE ANALYSIS OF COMPOUNDS LEACHED FROM DIFFERENT CLEAR ALIGNER MATERIALS: AN IN-VITRO STUDY

Dissertation submitted to

SANTOSH DEEMED TO BE UNIVERSITY, GHAZIABAD, NCR DELHI

In partial fulfillment of the requirement for the degree of

MASTER OF DENTAL SURGERY

In the Speciality of

ORTHODONTICS & DENTOFACIAL ORTHOPEDICS

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ACKNOWLEDGEMENTS

"Education is not the learning of facts but training the mind to think."

- Albert Einstein

First of all, I take this opportunity to express my profound gratitude and thanks towards my esteemed Head Of Department, Dr. Rajiv Ahluwalia, Professor, Department of Orthodontics and Dentofacial Orthopedics, Santosh Dental College and Hospitals, Santosh Deemed to be University, Ghaziabad, NCR Delhi, for constant support that pushed me to do better in the course and also in this dissertation. His professional advice, expertise and positive criticism enabled me to improve. With great sense of gratitude, I want to thank him for his generous and constant encouragement during my course. I shall forever remain indebted to him for the over whelming help and meticulous care taken in successfully completing this work.

I would like to express my special thanks of gratitude to my guide, Dr. Tina Chugh, Reader in the Department of Orthodontics and Dentofacial Orthopedics, Santosh Dental College and Hospitals, her guidance and advice carried me through all stages of writing my dissertation. I am absolutely grateful to her for the constant attention and professional guidance without which I couldn't have been a part of this academic accomplishment.

I would like to express my sincere gratitude to my co-guide Dr. Mayank Gupta, Reader in the Department of Orthodontics and Dentofacial Orthopedics, Santosh Dental College and Hospitals, for being a constant source of encouragement and wisdom. His insights

and expertise has enabled me to gain a deeper understanding of the subject matter.

His comments and suggestions were incredibly insightful and helped me to improve the quality of my work significantly.

I express my deep sense of gratitude to Dr. Nishant Gupta, Professor in the Department of Orthodontics and Dentofacial Orthopedics, Santosh Dental College and Hospitals for consistent encouragement, support supervision and suggestions throughout this dissertation. His valuable help was there at each step guiding me to prepare this dissertation. I am deeply grateful for his detailed and constructive comments, and for his important support throughout this work.

I take this opportunity to thank my college dean, Dr. Akshay Bhargava, MDS(Prostho)., AFAAID, DICOI, DWCOI., Santosh Dental College and Hospital, for his generous support and for being a constant driving force towards the completion of my dissertation.

I extend my heartiest thanks to my batch mates Dr. Shambhavi Rajput, Dr. Komal Garje, Dr. Nalin Yadav and Dr. Puja Kumari, for their support, continued guidance and advice throughout my Dissertation and co-operation at every step in my entire post graduate life. Thank you for being as backbone to steer out this work successfully.

I cannot be thankful enough to my lovely juniors Dr. Adithya Nair, Dr. Aditi Jain, Dr. Harshita Dabas, Dr. Priyanka Roy, Dr. Priyanka Thomas, Dr. Samreen Fatima Khan, Dr. Abhishek J Sai, Dr. Anjana Gopinath, Dr. Kankalina Hazra for always being there to help me.I would like to express my sincere thanks to my seniors, my mentors and my friends Dr. Palak Kulshreshtha, Dr. Shivangi Shukla, Dr. Anamika Sharma, Dr. Hemlata Bajaj

and Dr. Shikha Singh, for their never ending constant support and valuable suggestions.

I would like to express my sincere gratitude to Dr. Asma Sattar Khan, for providing the facilities and guidance necessary to carry out the experimental work in this study. I am also deeply thankful to Mr. Suryansh Kashyap, the chemical analyst, for their invaluable technical assistance and expertise in operating the GC-MS system and interpreting the analytical data. Their support was instrumental in the successful completion of this research.

My deepest gratitude goes to my family for their unconditional love and support throughout my life, this dissertation is simply impossible without them. Words are inadequate to express my feelings towards my father Dr. Barjinder Singh, my mother Dr. Richa Singh and my sister Amrita Singh, for their countless blessings, affection and moral support during this task. Their guidance, love and advice have been invaluable to me, and I am forever grateful for the efforts they have made for me.

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LIST OF ABBREVIATIONS

ABBREVIATION	FULL FORM
ANOVA	Analysis of Variance
BIS-GMA	Bisphenol A Glycidyl Methacrylate
BPA	Bisphenol A
CAD/CAM	Computer-Aided Design/Computer-Aided Manufacturing
CHDM	Cyclohexane Dimethanol
FAME	Fatty Acid Methyl Ester
FSL	Food stimulating liquid
HDA	Heptadecanoic Acid, 16-methyl, Methyl Ester
HEMA	Hydroxyethylmethacrylate
HPLC	High-Performance Liquid Chromatography
NIST	National Institute of Standards and Technology
PCTG	Polycyclohexylenedimethylene Terephthalate Glycol-Modified
PE	Polyethylene

PET	Polyethylene Terephthalate
PET - G	Polyethylene Terephthalate Glycol
PMMA	Polymethyl Methacrylate
PP	Polypropylene
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
TEGDMA	Triethylene Glycol Dimethacrylate
TPU	Thermoplastic Polyurethane
UDMA	Urethane Dimethacrylate

Introduction

INTRODUCTION

Orthodontic practice has seen significant technological growth over the years, with one of the most notable innovations being the introduction of transparent plastic materials in clear aligner therapy (CAT). Thermoplastic clear appliances have been used in dentistry for decades in various forms, such as retainers, night guards, splints for temporomandibular joint disorders, and bleaching trays.[1] Clear aligners are transparent, removable trays that fit over the teeth in the dental arch and apply a three-dimensional force to move specific teeth.As the patient moves from one aligner tray to the next, each tray is slightly different and gently pushes the teeth. Over time, these small changes help the teeth move slowly and steadily into the correct position.[2]

The success of clear aligner treatment depends on two main things: modern computer technology (CAD/CAM) and the ability of plastic materials to be easily shaped. Together, these help make the treatment more effective and more comfortable for the patient. The computer technology helps to design aligners with great accuracy, while the special plastics used can be molded to fit each patient's teeth perfectly. This combination allows teeth to move gradually and smoothly, leading to better results with less discomfort. The modern concept of clear aligners began in 1994 with the development of the ESSIX system by Sheridan,[3] whose work is based on Kesling's principles to fabricate retainers from thermoplastic sheets after fixed appliance therapy.[4] Initially used for retention, clear aligners took a major leap forward in the late 1990s with the creation of Align Technology© and its Invisalign® system, which revolutionized the market.[5]

Clear aligner material base components are polymers including polyethylene (PE), polyethylene terephthalate (PET), polyethylene terephthalate glycol (PETG), polyurethane (PU), and polypropylene (PP), polyethylene (PE), polycarbonate (PC).[6]

Clear aligner materials have progressed from initial single-layered or monophasic plastics to second-generation polyurethane-based compositions, and more recently to advanced third-generation multilayered designs. These modern materials typically feature a combination of soft and hard layers where the soft layer facilitates elastic deformation for better aligner adaptation, and the hard layer provides structural strength and long-term durability.

Polyethylene terephthalate (PET) and its modified form, polyethylene terephthalate glycol (PETG) an amorphous, non-crystallizing copolymer, are widely used in clear aligner manufacturing because of their superior mechanical strength and optical clarity. PET is synthesized from ethylene glycol and terephthalic acid and can exist in both amorphous and crystalline states, with its structural form significantly impacting its physical properties.

Enhancing the mechanical performance of these materials can be achieved by blending various polymers such as polyester, polyurethane, and polypropylene, an approach commonly adopted in the commercial production of aligners.

The specific ratio of polymers used in the blend, plays a crucial role in determining the material's final properties. For example, a PETG/polycarbonate (PC)/thermoplastic polyurethane (TPU) blend in a 70:10:20 ratio has demonstrated enhanced mechanical performance and the ability to maintain adequate orthodontic force over time compared to other ratios. Likewise, a PETG/PC2858 blend at a 70/30 ratio achieved an optimal balance of tensile strength, impact resistance, and

elongation at break, indicating its potential for reliable clinical use.[6]

Typically, clear aligners are made from high-quality thermoplastic materials through a thermoforming process, using conventional or digital dental models.[7]

Even though aligners are made very carefully and fit the teeth well at first, they don't keep their original shape or strength once they are worn in the mouth. As the patient wears them, the aligners slowly change because of the oral environment and the pressure from moving teeth. Over time, the material becomes weaker and less effective, and the aligners don't work as well to move the teeth. This is why patients need to switch to new aligners regularly during treatment.[8] Aligners must be worn nearly full-time about 22 hours a day except during eating and oral hygiene routines. Patients usually switch to a new set every two weeks and continue this process until treatment is complete.[9]

However, concerns have been raised regarding the biological safety of polyurethane, the material used in most clear aligners. During treatment, aligners remain in close contact with the oral environment for extended periods and are constantly exposed to fluctuating temperatures, moisture, respiration, bacteria, and salivary enzymes.[8] Additionally, they are subjected to mechanical stress from regular oral functions, including contact at cusp tips and habits like bruxism.[10]

Several studies have shown that prolonged exposure to certain clear aligner materials can negatively affect gingival cell health, increasing membrane permeability and disrupting cell-to-cell adhesion, which can compromise epithelial integrity and lead to microleakage.[11] In 2014, Premaraj et al. reported that transparent aligners could trigger a range of allergic reactions, from mild symptoms like a sore throat to more serious responses such as widespread skin rashes.[12]

Synthetic elastic polymers used in aligners can be challenging in how they perform clinically, when these materials are stretched too much and kept under steady pressure, they go through a process called load relaxation. This means the strong force they apply at first slowly weakens over time, reducing their ability to move teeth effectively. In clinical settings, this can result in a loss of 50% to 75% of the original tooth-moving force within just the first day. However, the exact mechanisms causing this time-dependent force decay remain unclear and may involve physical damage to the polymer, chemical breakdown, or a combination of both.[13]

Although commercially available polyurethane elastomers are generally thought to have acceptable stability in moist environments, their chemical structure includes ester and ether linkages, which make them more susceptible to hydrolysis. Specifically, in polyesterurethanes, degradation typically involves the hydrolysis of ester bonds, resulting in the formation of alcohols and carboxylic acids.[13]

In a related study on dental composites, which are also polymer-based materials,

Wu and McKinney (1982) found surface damage that was believed to be caused by softening and partial removal of the polymer matrix by chemicals present in the oral environment. While softening alone can weaken dental restorations, it also increases wear, especially on occlusal surfaces. This hypothesis was supported through wear tests on composite samples that had been conditioned in food-simulating liquids (FSLs), selected based on FDA recommendations from 1976. Some FSLs significantly attacked the polymer matrix, reducing the material's resistance to wear. This investigation was later expanded to include additional commercial composite materials and other polymer-based products.[14]

FSLs can be ranked for their softening effects using thermodynamic solution theory,

as proposed by Hildebrand and Scott 1950.[14] According to this theory, the maximum softening of a polymer is expected when the solubility parameter of the liquid closely matches that of the polymer itself. The solubility parameters of various solvents, including those relevant to FSL testing, can be found in the Polymer Handbook by Burrell, 1975.[14]

Concerns over the release of potentially harmful chemicals from dental appliances have increased in recent years. Vandenberg et al in 2007 in a study conferred that Bisphenols constituted the largest amount of these additive chemical[15] and were used to harden plastic and increase its clearance.[16] There are many different analogues of bisphenols and the main factor that determines the type of bisphenol, is the difference in the reactant component. The capital letter after the expression bisphenol is used to express the reactant component in it.[17] The most widely known derivative of bisphenols is Bisphenol A (BPA). More than 3.5 million tons of BPA are produced annually worldwide, and more than 100 tons of this amount are released into the environment.[18] It is possible to be exposed to bisphenols in different ways due to their wide range of uses and the long-term release from plastic products. There is always a concentration of bisphenol in body fluids, and exposure to BPA is mostly oral.[19]

In 2011, the World Health Organization identified bisphenol A (BPA) as an endocrine-disrupting chemical, drawing significant attention due to its estrogen-like effects.[20] BPA exposure has been linked to a range of health issues, including organ disruption, teratogenic effects, early puberty onset, feminization in males, and possible carcinogenicity. Animal studies have detected BPA in numerous body fluids, such as urine, fetal and adult blood, amniotic fluid, placental tissue, breast milk, and

saliva.[21]

Considering the widespread and growing use of polymer-based aligners in adult orthodontic treatment, understanding their chemical stability and biocompatibility is essential. Although these materials are widely considered safe and effective, their potential for degradation and leaching of chemical substances especially under constant exposure to intraoral conditions raises significant clinical and safety concerns. The possible release of BPA and other compounds, along with the mechanical and chemical stress encountered in the mouth, highlights the need for ongoing research into the long-term behavior of these materials.

This study aims to identify and compare the chemical compounds released from three distinct orthodontic aligner materials following accelerated ageing, induced through immersion in polar solvents such as ethanol, utilizing Gas Chromatography–Mass Spectrometry (GC-MS) analysis.

Aim & Objectives

AIM AND OBJECTIVES

AIM

To quantitatively determine and compare the compounds leached from 3 different types of Orthodontic Aligner materials using the Gas Chromatography–Mass Spectrometry unit (GC-MS).

OBJECTIVES

1. To determine the various chemical compounds leached from polyethylene (PE) aligner material after 7 and 14 days of immersion in ethanol.

2. To determine the various chemical compounds leached from polyethylene terephthalate-glycol (PET-G) aligner material after 7 and 14 days of immersion in ethanol.

3. To determine the various chemical compounds leached from polycyclohexylenedimethylene terephthalate glycol-modified (PCTG) aligner material after 7 and 14 days of immersion in ethanol.

4. To compare the chemical compounds leached from the three aligner materials over 7 and 14 days, in order to evaluate differences in their chemical stability.

RESEARCH HYPOTHESIS

The hypothesis tested in this study was that when exposed to an alcoholic medium the aligner materials undergo rapid degradation, which adversely affects their chemical properties and cause leaching.

NULL HYPOTHESIS

There is no difference in leaching of chemical substances among various aligner materials with respect to either type or quality of chemical substances.

Review of Literature

REVIEW OF LITERATURE

McKinney JE, Wu W (1985)[14] conducted a study aimed at investigating the effects of chemical food-simulating liquids on the wear resistance of various commercially available dental composite restorative materials. Specifically, the study aimed to assess how pre-conditioning in these liquids influenced the surface hardness and wear behavior of the composites. Four types of commercial dental composites were selected for testing: a conventional quartz-filled composite, a strontium-glass-filled composite, a visible-light-activated composite, and a microfilled composite. The specimens were pre-conditioned for one week in a range of chemical food-simulating liquids, including heptane and aqueous ethanol solutions with solubility parameters (δ) ranging from 1.5 to 4.8 × 10^4 $J^{1/2}m^{-3/2}$.) Pre-conditioning generally led to swelling of the polymer matrix and surface deterioration, particularly at solubility parameters near δ = 3 × 10^4 $J^{1/2}m^{-3/2}$ (equivalent to a 75% ethanol solution), where a significant reduction in surface hardness was observed across all composite types. In most cases, the increase in wear correlated with the decrease in hardness.

Vallittu PK, Miettinen V, Alakuijala P (1995)[22] conducted this study which aimed to quantify the content and amount of residual monomer released from heat-cured and chemical-cured polymethyl methacrylate (PMMA) during water storage. The research focused on understanding how curing methods and storage conditions influence the leaching behavior of monomers, which has implications for the biocompatibility of dental prosthetic materials. Residual monomers were extracted from ground PMMA

using tetrahydrofuran, while monomers released into storage water were collected with n-hexane. High-performance liquid chromatography (HPLC) was employed for the analysis. PMMA samples were stored in water for up to 8 weeks at two different temperatures: 22°C and 37°C. The amount of residual monomer release was monitored over time to assess both the extent and rate of leaching. Chemical-cured PMMA exhibited a higher residual monomer content compared to heat-cured PMMA. Storage temperature had a statistically significant effect on monomer release ($p < 0.001$). After the first day of storage at 37°C, the chemical-cured PMMA released 1906.7 ppm of monomer, while heat-cured PMMA released only 34.5 ppm. Study suggests that across the board, monomer release was greater at 37°C than at 22°C ($p < 0.001$).

Nathanson D, Lertpitayakun P, Lamkin MS et al (1997)[23] investigated the leachability of bisphenol A (BPA) and other components from dental sealants and composite materials. The study utilized seven commercially available light-cured pit and fissure sealants, including Delton, Prisma:Shield, and Concise, among others. The sealants were cured using a light-curing device, and samples were prepared by placing them in ethanol to extract leachable components. The extraction process involved measuring the weight of the sealants before and after exposure to the solvent. HPLC was used to separate the compounds, and the concentration of each component was calculated by integrating the absorption peaks of the chromatograms. The study found that the leachability of BPA was not detected in the tested sealants, contradicting previous reports. Instead, TEGDMA and BIS-GMA were identified as the major components leached from the sealants

The results indicated that the amount of leachable components varied significantly among different sealants, with Delton leaching the highest amounts of TEGDMA and BIS-DMA, while Prisma:Shield leached the least.

Eliades T, Eliades G, Watts DC (1999)[24] aimed to examine the structural characteristics of open and closed elastomeric modules in their original state and after various conditions: 24-hour in vitro loading at 50% extension, 24-hour intraoral exposure, and 3-week intraoral exposure. Sixty specimens (10 of each type from three brands) were analyzed. Evaluations were conducted using optical transmission microscopy (phase contrast and polarized light), micro MIR FT-IR spectroscopy, and scanning electron microscopy with X-ray microanalysis. After 24 hours intraorally, specimens had a protein-rich film with minimal phosphate presence, while 3-week specimens showed calcium phosphate deposits. The biofilm composition and calcification resembled non-specific adsorption seen in biomaterials exposed to body fluids. The results suggest that in vitro studies of elastomeric force decay should account for biofilm adsorption and calcification processes observed in vivo.

Suzuki K, Kazuomi I, Sugiyama K (2000)[25] aimed to quantify both the total and released amounts of bisphenol A (BPA) from polycarbonate-based dental materials, and to evaluate the effects of physical and thermal processing on BPA release. Orthodontic brackets and denture base resins were analyzed in their commercial form. Specimens were cut into standardized sizes and also crushed into powder.

High-pressure liquid chromatography (HPLC) was used to measure BPA release after immersion in water or ethanol for 1 hour. Total BPA content was determined after dissolution in chloroform. In ethanol, these values increased to 0.12–9.42 μg/g and 0.42–22.24 μg/g, respectively. The total BPA content in denture preform resin was 78.9 μg/g, rising to 90.2 μg/g after heat processing and up to 250 μg/g after mechanical crushing, indicating increased BPA availability due to polymer degradation. The findings confirm that BPA can leach from polycarbonate dental materials, and that manufacturing processes involving heat and mechanical stress can enhance BPA release.

Pulgar R, Olea-Serrano MF, Novillo-Fertrell A et al (2000)[26] aimed to evaluate the release of biphenolic compounds—including bisphenol A (BPA) and its derivatives—from Bis-GMA-based dental composites and sealants, both before and after in vitro polymerization. Seven commercial composites and one sealant were tested under controlled pH and temperature conditions. Samples were analyzed pre- and post-polymerization. The detection and quantification of leached components were conducted using high-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS). The study confirmed the leaching of estrogenic compounds, including BPA (up to 1.8 pg/mg), Bis-DMA (1.15 pg/mg), bisphenol A diglycidyl ether (6.1 pg/mg), Bis-GMA (2.0 pg/mg), and ethoxylated/propoxylated derivatives of BPA. These findings support concerns about the estrogenic potential of components released from Bis-GMA-based dental

materials.

Watanbe M, Hase T, Imai Y (2001)[27] investigated the leaching behavior and changes in bisphenol A (BPA) content of polycarbonate (PC) orthodontic brackets over time, given ongoing concerns about BPA's estrogenic potential in dental materials. PC orthodontic brackets were immersed in water at two temperatures (37°C and 60°C). At set intervals, the BPA content remaining in the brackets and the amount released into the water were measured using high-performance liquid chromatography (HPLC). BPA release increased over time. After 12 months at 37°C, the BPA concentration in water was 3.8 times higher than the initial level, and after 14 weeks at 60°C, it increased 12.4-fold. The rate of BPA release accelerated with time, indicating a time-dependent leaching process.

While the results confirmed that BPA leaches from PC brackets under simulated oral conditions, the study concluded that the levels released are unlikely to produce significant estrogenic effects in the human body.

Al-Hiyasat AS, Darmani H, Elbetieha AM (2004) examined the impact of leached components from a resin-based dental composite (Z-100) and bisphenol A (BPA) on female mouse fertility and reproductive outcomes. Female mice were administered either BPA (5, 25, or 100 µg/kg) or leachates from the Z-100 composite via intragastric dosing daily for 28 days. A control group received distilled water. Mice were then mated with untreated males, and fertility parameters were assessed. The chemical composition of the leachates was analyzed using high-performance liquid chromatography (HPLC). Exposure to Z-100 leachates

significantly reduced pregnancy rates (54.5%) compared to controls (100%), and increased relative ovarian weights by 142%. BPA exposure at 25 and 100 µg/kg led to significantly higher numbers of embryonic resorptions and increased uterine weights. High-dose BPA also increased ovarian weights. HPLC analysis identified tri-(ethylene glycol)-dimethacrylate (TEG-DMA) as the primary leached component (5945 µg/ml), followed by BIS-GMA (2097 µg/ml) and BPA (78 µg/ml).

The findings demonstrate that both BPA and composite leachates adversely affect female fertility and reproductive health in mice.

Reichl FX, Esters M, Simon S et al (2006)[29] investigated whether components released from dental restorative materials could reach cytotoxic levels in human oral tissues by evaluating the toxicity of several resin-based dental (co)monomers in comparison to known mercury compounds. The study tested hydroxyethylmethacrylate (HEMA), triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), and bisglycidyl methacrylate (BisGMA), alongside methyl mercury chloride (MeHgCl) and mercuric chloride ($HgCl_2$), on cultured human gingival fibroblasts (HGF). Two cytotoxicity assays were employed: a modified XTT assay and a modified Hoechst 33342 staining method. The relative toxicities ranked from lowest to highest were: HEMA < TEGDMA < UDMA < BisGMA < $HgCl_2$ < MeHgCl. Mechanistically, TEGDMA and MeHgCl primarily induced apoptosis, whereas HEMA, UDMA, BisGMA, and $HgCl_2$ caused predominantly necrotic cell death.

Gioka C, Bourauel C, Hiskia A et al (2005)[30] conducted a study to evaluate the degree of cure and monomer leaching of a light-cured and a chemically cured (no-mix) dental adhesive, alongside assessing their biological effects. The degree of polymerization was determined using infrared spectroscopy on samples prepared to simulate clinical application. These adhesives were immersed in normal saline for two months, after which the quantity and identity of residual monomers were analyzed using liquid chromatography. The biological impact of the eluents was tested on human gingival fibroblasts using MTT and DNA synthesis assays to assess cell viability and proliferation. Statistical analysis included ANOVA and the Tukey test ($\alpha = 0.05$). The study found no significant difference between the two adhesives in terms of degree of cure or the amount of triethylene glycol dimethacrylate (TEGDMA) released, and diglycidyl dimethacrylate (Bis-GMA) was not detected in any sample. Neither adhesive showed cytotoxicity, although both caused a moderate decrease in DNA synthesis, indicating a minor cytostatic effect. The findings suggest that while the adhesives are not overtly harmful to fibroblasts.

Eliades T, Eliades G, Silikas N (2005)[31] investigated the structural characteristics and degradation behavior of orthodontic polyurethane elastomeric modules subjected to mechanical and chemical aging. The study evaluated three commercial products (Generation II, Dentaurum, and Alastic), each divided into four experimental groups: as-received (A0), mechanically aged by 3 weeks of stretching at 50% elongation in air (B0), chemically aged by immersion in an ethanol/water solution (3:1 v/v) for 3 weeks (A1), and combined mechanical and chemical aging under the same solution and elongation (B1). Structural analysis of the elastomeric

chains and their solution extracts was conducted using micro-MIR FTIR spectroscopy. The B0 group exhibited changes in IR peak intensities compared to A0, indicating molecular orientation of poly(methylene glycol) soft segments and degradation of α-methylene groups, suggestive of oxidative processes forming esters, carboxylic acids, or aldehydes. While the spectra of groups A1 and B1 were largely similar to controls, a notable disappearance of the 1639 cm^{-1} peak associated with stearamide wax was observed. The findings suggest consistent structural degradation patterns across all tested products, indicating potential for similar degradation mechanisms in the oral environment.

Eliades T, Bourauel C (2005)[32] conducted a systematic editorial analysis exploring the impact of various aging factors on the morphology, structure, and mechanical properties of polymeric and metallic orthodontic materials. The study assessed how aging affects the longevity of bond strength and orthodontic mechanotherapy. The study also examined how aging alters force transmission between archwires and brackets and affects friction during sliding mechanics. The editorial concludes by emphasizing the need for orthodontic materials research to adopt clinically meaningful test methodologies and to clearly define the limitations of laboratory models to accurately interpret clinical relevance.

Eliades T, Hiskia A, Eliades G (2007)[33] conducted a study aimed at quantitatively characterizing the release of bisphenol-A (BPA) from orthodontic adhesives after artificial accelerated aging. Two types of adhesives, a chemically cured no-mix adhesive and a visible light-cured adhesive, were each bonded to 20 stainless steel

brackets, forming two groups. Three sets of specimens were prepared for each adhesive group. All samples were immersed in alcohol to induce accelerated aging, and eluents were collected after 1 day, and at 1, 3, and 5 weeks. These samples were analyzed using high-performance liquid chromatography (HPLC), with all tests performed in triplicate and results averaged. No trace of BPA was detected in any sample at any time point, indicating that, if present, its concentration was below the HPLC detection limit of 0.1 ppm (0.1 μg/L). The study concluded that both light-cured and chemically cured no-mix adhesives did not release detectable amounts of BPA under the test conditions. It recommended conducting estrogenicity assays to better understand potential hormonal effects and suggested developing adhesives formulated with high molecular weight, benzoic ring-free monomers to mitigate concerns associated with Bis-GMA.

Gracco A, Mazzoli A, Favoni O et al (2009)[10] conducted a study to investigate the short-term optical, chemical, and morphological changes occurring in Invisalign appliances. The research included one unused aligner, one unused aligner immersed in artificial saliva for 14 days, and 10 aligners worn by different patients over a 14-day period. A combination of analytical techniques was used: Fourier transform infrared microspectroscopy to detect surface molecular changes, spectrophotometry for assessing alterations in color and transparency, scanning electron microscopy and energy-dispersive X-ray microanalysis to observe surface morphology and elemental composition of deposits, and gas chromatography-mass spectrometry to identify substances leached into artificial saliva. Aligners worn for 14 days exhibited microcracks, surface abrasion, delamination, localized calcified biofilm, and

decreased transparency. No monomers or degradation by-products were detected in the aligner immersed in artificial saliva, indicating chemical stability of the material under those conditions.

Eliades T, Pratsinis H, Athanasiou AE (2009)[34] conducted an in-vitro study to evaluate the cytotoxic and estrogenic properties of Invisalign appliances (Align Technology, Santa Clara, Calif). Three complete sets of maxillary and mandibular as-received aligners were immersed in normal saline solution for two months. The eluents were then diluted to concentrations of 5%, 10%, and 20% (vol/vol) and tested for cytotoxicity on human gingival fibroblasts and for estrogenic potential using the proliferation rate of MCF-7 estrogen-sensitive breast cancer cells. Each assay was performed four times per appliance set, and results were statistically analyzed using two-way ANOVA, with appliance and concentration as predictors, and post-hoc comparisons made with the Tukey test at a 0.05 significance level. No cytotoxic effects on gingival fibroblasts or estrogenic stimulation of MCF-7 cells were observed at any concentration, suggesting that Invisalign appliance eluents do not exhibit estrogenicity under the tested conditions.

Kang Y-G, Kim J-Y, Kim J et al (2011)[35] conducted a study to evaluate changes in bisphenol A (BPA) levels in saliva and urine following the placement of lingual bonded retainers. Using liquid chromatography/mass spectrometry, BPA concentrations were measured in samples collected from 22 volunteers at multiple time points: before placement, and 30 minutes, 1 day, 1 week, and 1 month post-placement. The effects of resin type (nanohybrid-filled flowable resin vs.

conventional hybrid resin), surface prophylaxis, age, and sex on BPA levels were also analyzed. A significant increase in BPA was observed only in saliva samples collected immediately after retainer placement. The maximum BPA concentration detected (20.889 ng/mL) was well below the established reference daily intake. The study concluded that BPA release from lingual retainers is minimal and unlikely to pose toxicological risks.

Sunitha C, Kailasam V, Padmanabhan S (2011)[36] investigated the release of bisphenol A (BPA) from an orthodontic adhesive (Transbond XT; 3M Unitek) when cured at varying distances from the light-curing tip and assessed the correlation with the degree of conversion. Using high-performance liquid chromatography, BPA release was measured from 138 brackets divided into groups based on curing distances of 0, 5, and 10 mm. The degree of monomer conversion was analyzed via Fourier transform infrared spectroscopy. Statistical analysis included two-way ANOVA, Tukey HSD post-hoc tests, paired t-tests, and Pearson correlation. The results showed that greater light-curing distances led to increased BPA release and a reduced degree of conversion. A negative correlation was observed between BPA release and monomer conversion. The findings suggest that to minimize BPA release, clinicians should maintain minimal distance between the curing light and adhesive during bonding procedures.

Rathee M, Malik P, Singh J (2012)[37] conducted a review in which they discussed the use and potential biological effects of Bisphenol A (BPA) in dental materials. BPA-based epoxy resins are commonly used in various commercial products,

including dental resins, polycarbonate plastics, and food can linings. In dentistry, BPA serves as a precursor to Bis-GMA, a resin monomer widely used in sealants and restorative materials. BPA may be present in these products either as a residual impurity or as a degradation by-product of Bis-DMA, facilitated by enzymatic activity in saliva. Leaching of BPA and related monomers from dental resins can occur during the initial polymerization phase. These leached compounds may be bioactive. Studies have demonstrated that BPA, Bis-DMA, and Bis-GMA exhibit estrogenic activity, raising safety concerns due to BPA's ability to bind non-specifically to estrogen receptors. The review highlights the potential endocrine-disrupting effects of BPA and related compounds in dental sealants and underscores the need for careful evaluation of their biocompatibility and long-term effects.

Kloukos D, Pandis N, Eliades T (2013)[38] conducted this systematic review aimed to evaluate the short and long-term release of chemical components from orthodontic adhesives and polycarbonate brackets in the oral environment. An extensive search of both published and unpublished literature was conducted across multiple databases, including medline, embase, central, and others, without restrictions on language or publication date. No randomized controlled trials were found; therefore, the review included eleven observational studies conducted either in vivo or in vitro. These studies reported a wide range of bisphenol A (BPA) release from orthodontic bonding resins, with levels ranging from 0.85 to 20.88 ng/mL in vivo and up to 65.67 ppm in vitro. Polycarbonate brackets were found to release 22.24 mg/g in ethanol solution and up to 697 mg/g after 40 months of water exposure. In vitro leaching of other monomers such as Bis-GMA and TEGDMA reached

concentrations of 64 mg/10 mL and 174 mg/10 mL, respectively. Overall, the current evidence comes from observational studies, offering a moderate level of confidence, but highlights the need for standardized methodologies and higher-quality trials to draw more definitive conclusions.

Kotyk MW, Wiltshire WA (2014)[39] conducted a study, objective of this study was to quantitatively determine the amount of bisphenol-A (BPA) leached from orthodontic materials under simulated intraoral conditions. Orthodontic samples were subjected to various conditions, including simulated abrasion, immersion in artificial saliva, thermal shock from temperature cycling, and other simulated intraoral exposure. Samples were collected for up to two weeks following immersion in artificial saliva, derivatized, and analyzed for BPA using gas chromatography/mass spectrometry. The results indicated that measurable amounts of BPA leached from both a thermoformed orthodontic retainer material (7.63 mg/g) and an orthodontic adhesive (2.75 mg/g). The leaching of BPA was observed only within the first three days of immersion in artificial saliva. Although the quantities of BPA leached were below the reference dose for daily intake, the study suggests that due to the potential low-dose effects and the association of elevated urinary BPA levels with various medical disorders, exposure to BPA should be minimized or eliminated, especially from materials that demonstrate leaching.

Premaraj T, Simet S, Beatty M et al (2014)[12] aimed to examine the biological impact of Invisalign plastic aligners on oral epithelial cells in vitro, specifically looking at how the cells respond when exposed to substances leached from the

aligner material. Epithelial cells were exposed to eluates obtained by soaking Invisalign plastic in either saline or artificial saliva for 2, 4, and 8 weeks. Control groups were maintained in plain saline or saliva-based media. Morphological changes were observed using light microscopy, while cell viability and membrane integrity were evaluated using MTT assays and flow cytometry. The results showed that cells exposed to saline-derived eluates experienced significant morphological changes, decreased viability, and impaired membrane integrity, indicating cytotoxicity. In contrast, eluates from artificial saliva did not produce significant adverse effects. ECIS results further confirmed reduced adhesion and mobility in cells treated with saline eluates.

Ahn H-W, Ha H-R, Lim H-N (2015)[40] investigated the effect of thermoforming and intraoral exposure affect the physical and chemical properties of thermoplastic vacuum-formed retainers (VFRs), particularly those made from 0.8-mm PETG sheets. Using samples from 48 patients over two durations (2 weeks and 6 months), the researchers employed multiple analytical techniques, including Light Microscopy (LM), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Raman spectroscopy, Energy-Dispersive X-ray spectroscopy (EDX), and mechanical testing. Findings revealed that prolonged intraoral exposure significantly altered the surface morphology and molecular composition of VFRs. Mechanical tests indicated increased tensile strength, elastic modulus, and energy absorption with longer exposure times. While thermoforming produced smoother, non-crystallized PETG surfaces, intraoral aging accelerated structural and mechanical changes, with effects varying by location. The results emphasize that

both thermoforming and oral environment exposure have significant, time-dependent impacts on the performance and durability of VFRs.

Thavarajah R, Thennukonda RA (2016)[41] examined the adverse events and reactions (AERs) associated with sequential aligners (SAs), using data from the FDA's Manufacturer and User Facility Device Experience (MAUDE) database over a five-year period. Despite the widespread adoption of SAs in orthodontic treatment, limited data exists on their potential side effects. The authors analyzed 175 reported AER cases, of which 73.71% were mandatory manufacturer reports. Allergic reactions were among the most common, with 18.29% of cases involving suspected allergies, including 11.43% with anaphylaxis and 2.29% with angioedema. Intraoral symptoms such as inflammation of the tongue, throat, and lips were frequently reported, along with systemic issues like nausea, gastrointestinal distress, neuromuscular symptoms, headaches, coughing, fever, and even cardiac events. The findings underscore the need for heightened awareness of potential adverse effects associated with aligner therapy and call for more comprehensive, prospective research to better understand these risks.

Ansari S, Chaudhari A, Patil C (2016)[42] explored a simple yet effective method to evaluate the leaching potential of thermoplastic sheets used in clear aligner therapy, addressing the growing concern about their long-term biocompatibility. With increasing market availability of new materials, standardized testing remains limited. The researchers utilized a redox reaction involving two concentrations of potassium permanganate to test five different thermoplastic samples. The sequence of color

change—ranging from purple to clear—served as an indicator of leaching, with the reaction reflecting the stoichiometric balance between the oxidizing agent and the sample's reducing potential. Color changes were monitored visually over 24 hours, and photographic documentation was done at 15 minutes, 8 hours, and 24 hours using a Canon DSLR1000 camera. The results showed that diluted potassium permanganate accelerated color change due to its lower reagent concentration. Among the samples, 3A Medes (Korea) exhibited the highest leaching, followed by CA (Scheu, Germany), EVA (Endent, India), Ultradent (USA), and Avac R (Jaypee, India). The study concludes that this "do-it-yourself" redox test is a reproducible, chairside-compatible method that can be adapted for clinical screening of aligner material biocompatibility.

Walele A, Chaudhari PS, Patil C et al (2016)[43] evaluated the biocompatibility of thermoplastic sheets used in clear aligner therapy by quantitatively analyzing the organic compounds they release. The researchers examined thermoplastic sheets from five manufacturers: CA (Scheu, Germany), Ultradent (USA), 3A Medes (Korea), Avac R (Jaypee, India), and EVA (Endent Pvt. Ltd., India). Uniform samples were powdered using liquid nitrogen and eluted in an ethanol-water solution (75:25) at 35.5°C for seven days. The resulting extracts were analyzed using High-Performance Liquid Chromatography (HPLC) to quantify the leached organic compounds. The results indicated that Ultradent had the highest leaching potential, suggesting the lowest biocompatibility, whereas Avac R (Jaypee) showed the least amount of leaching, indicating greater compatibility with biological tissues. The study concludes that HPLC is an effective method to assess the leaching behavior of

aligner materials, providing insights into their safety and suitability for long-term intraoral use.

Raghavan AS, Sathyanarayana HP, Kailasam V (2017)[44] aimed to evaluate and compare the levels of bisphenol A (BPA), a known endocrine-disrupting compound, in the saliva of patients using different types of orthodontic retainers during the retention phase. Forty-five patients who had completed fixed orthodontic treatment were randomly divided into three groups: those using vacuum-formed retainers (VFRs), Hawley retainers fabricated with heat-cure acrylic, and Hawley retainers fabricated with chemical-cure acrylic. Salivary samples were collected at four time points: before appliance insertion, within one hour, after one week, and after one month. BPA levels were analyzed using two-way ANOVA and Tukey HSD post-hoc tests. The results revealed a significant rise in BPA levels across all groups post-placement ($P \leq 0.05$), with the highest concentrations observed in the VFR group, followed by chemically cured Hawley retainers. The heat-cured Hawley retainers showed the lowest BPA release. The study concludes that material composition and polymerization method significantly affect BPA leaching, advocating heat-cured Hawley retainers as the most biocompatible choice among the tested options.

Moreira MR, Matos LG, de Souza ID et al (2017)[45] investigated the release of bisphenol A (BPA) from five orthodontic composites, both in vitro and in vivo. The composites tested included Eagle Spectrum, Enlight, Light Bond, Mono Lok II, and Transbond XT. To simulate oral conditions, each material was immersed in a water-ethanol solution, and BPA release was measured at intervals of 30 minutes, 24 hours,

1 day, 1 week, and 1 month using gas chromatography coupled with mass spectrometry. Among the materials, Enlight showed the highest BPA release after one month, while Light Bond released the least. In the in vivo component, 20 patients undergoing fixed orthodontic treatment provided saliva and urine samples before bracket bonding and at multiple time points afterward. BPA levels in saliva showed a significant increase only at 30 minutes post-bonding, returning to baseline at subsequent intervals. Urine samples revealed a similar pattern, but the levels remained below the threshold for daily safe intake. Although all composites released BPA, the exposure was brief and within clinically acceptable limits, highlighting the importance of choosing materials with lower leaching potential for patient safety.

Naqbi SRA, Pratsinis H, Kletsas D (2018)[46] conducted this in vitro study aimed to evaluate the cytotoxic and estrogenic potential of Vivera® retainers, both in their original state and after clinical use. Six sets of retainers—three as-received from the manufacturer and three retrieved after four weeks of intraoral use were immersed in normal saline for 14 days, undergoing various sterilization procedures. The biological impact of eluates from these retainers was assessed using two distinct breast cancer cell lines: MCF-7. Cells were exposed to test solutions at concentrations ranging from 5% to 20% (v/v) in estrogen-depleted medium, and proliferation was measured after six days to assess estrogenic activity, with β-Estradiol serving as a positive control. The results were analyzed using two-way ANOVA, with retainer type and concentration as variables, followed by Tukey's post hoc test to determine significance at the 0.05 level. The findings contribute to the growing body of evidence regarding the biological safety of thermoplastic

orthodontic appliances.

Martina S, Rongo R, Bucci R (2019)[47] conducted this in vitro study and evaluated the cytotoxic effects of four commonly used thermoplastic materials for clear aligners Duran, Biolon, Zendura, and SmartTrack on human primary gingival fibroblasts (HGFs). Duran, Biolon, and Zendura were tested in both thermoformed and non-thermoformed states, while SmartTrack was assessed only in its thermoformed form. All samples were incubated in Dulbecco's Modified Eagle's Medium (DMEM) at 37°C for 14 days, and the resulting eluates were used to culture HGFs. Cell viability was measured using the MTT assay. The results indicated a mild cytotoxic effect across all materials, with Biolon showing the greatest reduction in cell viability (64.6%), followed by Zendura (74.4%), SmartTrack (78.8%), and Duran (84.6%), which was the least cytotoxic. Thermoforming significantly increased the cytotoxicity of Duran, Biolon, and Zendura. These findings suggest that while all materials tested exhibit some degree of cytotoxicity, the extent varies by material and is exacerbated by the thermoforming process.

Ihssen BA, Willmann JH, Nimer A (2019)[48] conducted an in vitro study to assess how the mechanical properties of orthodontic aligners made from polyethylene terephthalate glycol (PETG) degrade under simulated intraoral conditions. Sixty PETG specimens from CA Clear Aligner (Scheu Dental, Germany) were divided into three groups: water-immersed, thermocycled, and control. Each group was further tested at two different temperatures (22 °C and 37 °C) to simulate environmental and intraoral conditions. The study evaluated Young's modulus, 0.2%

offset yield strength (Rp02), and ultimate tensile strength (UTS). Additionally, water absorption was measured to understand the material's behavior under moisture exposure. he results showed significant reductions in mechanical properties at higher temperatures, with thermocycled specimens absorbing 48% more water than those only immersed. Young's modulus and UTS were consistently lower at 37 °C across all groups, and both treated groups demonstrated decreased stiffness compared to the control. These findings support the clinical observation that aligners lose effectiveness over time. The study concludes that environmental stressors like temperature fluctuations and moisture exposure compromise the aligner's elasticity, leading to a decline in the orthodontic forces they generate during treatment.

Iliadi A, Koletsi D, Papageorgiou SN (2020)[49] published a systematic review aimed to evaluate the safety of thermoplastic orthodontic appliances, such as aligners and retainers, focusing on their potential estrogenic and cytotoxic effects as well as the release of bisphenol-A (BPA) and other monomers. A comprehensive search of eight electronic databases yielded 58 studies, of which five met inclusion criteria—four were in vitro studies and one was a randomized controlled trial. The included studies examined thermoplastic materials either as-received or after intraoral use. Meta-analysis of two studies showed that eluents from these appliances did not promote MCF-7 cell proliferation, suggesting a lack of estrogenic activity at concentrations of 5% and 20% v/v. No cytotoxic effects were reported. While in vitro studies showed no detectable levels of BPA or monomer release, the clinical trial indicated a rise in BPA levels in saliva within 30 days of using thermoplastic retainers, in contrast to Hawley retainers. However, the overall quality of the

evidence was rated low to moderate due to high or unclear risk of bias. The current body of research suggests that thermoplastic appliances do not exhibit cytotoxic or estrogenic effects in vitro, but there is inconsistent evidence regarding BPA release, particularly under clinical conditions.

Katras S, Ma D, Dayeh AA (2021)[50] in this study investigated the release of Bisphenol A (BPA), a known endocrine disruptor associated with various health concerns, from three popular orthodontic aligner brands: SmileDirectClub®, Invisalign®, and Essix Ace®. To quantify and compare BPA leaching, equal-sized aligner samples were submerged in artificial saliva, artificial gastric fluid, and ethanol, then incubated at 37°C. Samples were periodically collected and analyzed using high-performance liquid chromatography tandem mass spectrometry. BPA release was observed in all three aligner types, with the highest concentrations detected in artificial saliva. Essix Ace® exhibited the highest release (6.3 ng/mL after 10 days), followed by SmileDirectClub® (5.0 ng/mL after 20 days), and Invisalign® (3.5 ng/mL after 24 hours). Although there were differences in timing, no statistically significant difference in BPA levels was found among the brands or between the media types. However, BPA release was significantly higher in the first 24 hours of incubation compared to baseline ($p<0.001$). These findings suggest that orthodontic aligners do release BPA, especially within the first day of use, though the total levels and differences between brands remain relatively small.

Dalaie K, Fatemi SM, Ghaffari S (2021)[51] examined how thermoforming and artificial aging affect the thermomechanical properties of two commonly used polyethylene terephthalate glycol (PETG) aligner materials—Duran and Erkodur. A total of 96 samples were divided into three groups.. Thermoforming was done using 3D-printed molds, and aging was simulated through 200 thermal cycles following immersion in 37°C distilled water for 24 hours. The study evaluated key mechanical parameters including flexural modulus, hardness, glass transition temperature (Tg), and dynamic mechanical properties (elastic modulus, viscous modulus, and loss factor) at different temperatures. Results showed that thermoforming significantly reduced flexural modulus (by 88% in Duran and 70% in Erkodur) and hardness (22% and 7.6%, respectively), while aging had minimal additional impact. Both materials exhibited a significant drop in Tg after thermoforming. Dynamic mechanical analysis revealed increased viscous modulus and loss factor post-thermoforming in both materials, with no significant changes due to aging. Duran consistently demonstrated better thermomechanical stability compared to Erkodur. Overall, the findings suggest that thermoforming plays a more critical role than aging in altering the functional properties of PETG aligner materials, which may influence their long-term clinical performance.

Hassan R, Aslam Khan MU, Abdullah AM et al (2021)[52] highlighted the growing significance of polymeric materials, especially thermoplastics, in orthodontics due to their adaptability and wide biomedical applications. Thermoplastic polymers are increasingly favored for their use in clear aligners and retainers, offering aesthetic and functional benefits. This study synthesizes recent advancements in BPA-free

monomers used in dental resins and adhesives, with a focus on emerging smart polymeric materials that demonstrate improved safety profiles and application potential in future orthodontic treatments.

Arslan B, Yıldırım E, Bodur OC et al (2022)[53] evaluated the influence of common oral hygiene practices specifically tooth brushing and mouth rinsing on the release of bisphenol A (BPA) from orthodontic adhesive materials. The investigation involved three experimental groups of adhesive samples: the first group served as a control, with standard polymerization; the second group underwent brushing with fluoride toothpaste post-polymerization; and the third group was exposed to an alcohol-based mouth rinse. All samples were subsequently stored in distilled water, and BPA concentrations were quantified using high-performance liquid chromatography (HPLC). The results revealed that all samples released detectable levels of BPA, with the highest release observed in the group exposed to the mouth rinse. Although the absolute BPA concentrations remained below established toxic thresholds, statistical analysis showed a significant increase in BPA release in the mouthwash group compared to the control ($p < 0.01$). These findings suggest that alcohol-based mouth rinses may accelerate BPA leaching due to the solvent properties of alcohol, underscoring the need for clinicians to consider the interaction between orthodontic materials and oral hygiene agents when advising patients.

Aseel Alhendia, Rita Khounganianb, Abdulazez Almudh (2022)[1] investigated and compared the cytotoxic potential of four commercially available clear aligner systems—Invisalign, Eon, SureSmile, and Clarity by assessing their effects on

human gingival fibroblasts. To simulate intraoral exposure, three sets of aligners from each system were immersed in normal saline at 37°C for a period of one month. The resulting solutions were then diluted to three concentrations (5%, 10%, and 20%) and applied to cultured fibroblasts for 48 hours. Following exposure, cell viability was assessed using the MTT assay, which quantifies mitochondrial activity as an indicator of cytotoxicity. While the comparison among the four aligner systems did not yield statistically significant differences, all tested materials exhibited a notable reduction in cell viability at higher solution concentrations (10% and 20%) compared to the untreated control. Among the aligners, Clarity showed the lowest overall cytotoxicity across all tested concentrations. These findings reinforce previous research suggesting that thermoplastic materials used in orthodontic appliances may release substances capable of affecting cell viability. Although the observed cytotoxicity was generally slight to moderate, the results highlight the importance of continued evaluation of aligner materials, especially as their prolonged intraoral use may contribute to cumulative biological effects.

Alhendi A, Khounganian R, Almudhi A (2022)[54] conducted an in-vitro investigation in which they aimed to evaluate and compare the chemical leaching potential of four clear aligner systems—Invisalign®, Eon®, SureSmile®, and Clarity®. Three aligners from each manufacturer were segmented and immersed in five solvent conditions, including 100% ethanol, 75% ethanol with 25% water, 50% ethanol with water, 25% ethanol with 75% water, and pure distilled water. These samples were incubated at 37 °C for two weeks, after which the eluents were analyzed using gas chromatography-mass spectrometry (GC-MS) to detect and

confirm any leached compounds.

The analysis identified a total of eleven chemical substances across the aligner brands, with Benzene 1,3-bis(1,1-dimethylethyl) consistently present in all systems at the higher ethanol concentrations (100% and 75%). While overall differences in leaching between systems were statistically insignificant, the Eon® aligners demonstrated a significantly higher number of leached substances across different immersion conditions compared to the others. Notably, Invisalign® showed the lowest leaching profile among the tested systems.

Willi A, Patcas R, Zervou S-K et al (2022)[55] conducted a study aimed to quantitatively evaluate the degree of polymer conversion and the leaching of specific chemical compounds from 3D-printed orthodontic aligners. The aligners, fabricated using Tera Harz TC85A photopolymer resin, were examined for their molecular composition and degree of monomer conversion through attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy. The degree of conversion was found to be approximately 83%, suggesting a relatively efficient polymerization process. To assess chemical leaching, aligners were immersed in double distilled water at 37°C for one week, and the eluates were analyzed using advanced liquid chromatography coupled with mass spectrometry. While bisphenol-A (BPA) was not detected in any samples, all aligners released quantifiable levels of urethane dimethacrylate (UDMA), with concentrations ranging from 29 to 96 µg/L. These findings suggest that although 3D-printed aligners made with this specific resin do not contribute to BPA exposure, they may release other potentially harmful compounds such as UDMA.

Yazdi M, Daryanavard H, Ashtiani AH (2022)[56] conducted a systematic review aimed to assess the biological safety and biocompatibility of clear aligners and vacuum-formed thermoplastic retainers, particularly concerning bisphenol-A (BPA) release. In response to growing concerns and conflicting findings in the literature, a comprehensive search was conducted across multiple databases including Web of Science, PubMed, Cochrane, Scopus, and Google Scholar, up to December 22, 2021. Studies of all types, languages, and designs were included if they addressed the biocompatibility, chemical safety, or estrogenic/cytotoxic properties of clear aligners or retainers. Studies focusing solely on mechanical properties were excluded. A total of 16 relevant studies were included in the review, comprising 1 randomized clinical trial and 15 in vitro experiments. BPA release was quantitatively reported in four studies. In vitro studies generally indicated minimal to no BPA release. Beyond chemical leaching, various adverse effects associated with the use of clear aligners and thermoplastic retainers were reported. In conclusion, while in vitro evidence supports a low risk of BPA exposure from clear aligners and retainers.

Shah J, Nithyashri M, Sonali Mahadevia B (2022)[57] conducted this study to develop a straightforward and practical method to assess the leaching potential of thermoplastic materials used in clear aligners. The study involved testing four different clear aligner brands: Scheu (Germany), Invisalign (USA), Flash Aligners (India), and Clear Correct (Switzerland). The method used to assess leaching involved a redox reaction between potassium permanganate and polymers from the clear aligner sheet samples. The color change sequence from purple to clear indicated the amount of leaching, which was measured based on the stoichiometric

relationship between the oxidizing agent (potassium permanganate) and the reducing polymer sample. The color change was observed at time intervals of 15 minutes, 8 hours, and 24 hours, and photographs were taken to document the changes. The results showed that the diluted solutions exhibited a faster color change, as the lesser amount of reagent accelerated the reaction. Scheu (Germany) was the first to show significant leaching, followed by Invisalign (USA), Flash Aligners (India), and Clear Correct (Switzerland). The study demonstrated that different aligner materials exhibited varying degrees of leaching, with Scheu leaching the most and Clear Correct leaching the least.

Çifçi Özkan E, Dumanlı Gök G (2023)[58] conducted a study, the objective of which was to evaluate the bisphenol release from different brands of clear aligner materials. Six clear aligner materials were tested: Duran (Scheu-Dental GmbH, Germany), Zendura Flx (Bay Materials LLC, USA), Zendura A (Bay Materials, USA), Essix (Raintree Essix Inc., USA), Taglus Premium (Laxmi Dental Export Pvt. Ltd, India), and Smart Track (Align Technology, USA). The samples were immersed in saline solution for 8 weeks in airtight test tubes at 37°C, with the sample-to-solution ratio set at 0.1 g/mL. Liquid chromatography-mass spectrometry/mass spectrometry analysis was conducted to assess bisphenol release. The results revealed that Bisphenol A (BPA) levels in Smart Track were significantly higher than those in Zendura A and Zendura Flx. In conclusion, while the amounts of leached BPA, BPF, and BPS were lower than the reference dose for daily intake.

Iliadi A, Zervou S-K, Koletsi D (2024)[59] conducted a study aimed to evaluate the impact of clear aligners on the surface characteristics and degradation of composite attachments, focusing on the release of resin monomers and their derivatives in an aqueous environment. Two types of composite materials—universal (C) and flowable (F)—were used for bonding to zirconia tooth-arch frames and thermoformed PET-G aligners. The aligners with bonded attachments were immersed in water for 7 days, simulating intraoral conditions with four daily cycles of removal and re-seating. The surface properties, roughness, and composition of the attachments were assessed before and after immersion. Eluents were analyzed using LC-MS/MS to detect common resin monomers (Bis-GMA, UDMA, 2-HEMA, TEGDMA, and BPA) and degradation products. The results showed that both composite materials experienced wear and damage, with flowable composites (F) showing greater morphological changes. While significant changes in the leached compounds were observed between Day 1 and Day 7, no significant differences were found between the two composite types.

Stocker L, Zervou S-K, Papageorgiou SN (2024)[60] aimed to investigate the release of substances from polyester-urethane (Invisalign™) aligners and resin composite attachments (Tetric EvoFlow) in vivo. Eleven patients who had been treated with the aligners and attachments for an average of 20 months participated. Prior to attachment removal, patients rinsed with 50 mL of distilled water, and the rinsing solution was collected. After the attachments were removed using low-speed tungsten carbide burs and adhesive residues were cleaned, a second rinsing was performed, and the solution was collected for analysis. The collected rinsing

solutions were analyzed using both targeted and untargeted methods. The targeted analysis, using LC-MS/MS, detected compounds such as Bis-GMA, DCDMA, UDMA, and BPA, while untargeted analysis through LC-HRMS screened for additional leached substances and their degradation products. The results showed a significant reduction in BPA levels after attachment removal, with BPA levels being four times lower post-removal. Bis-GMA, DCDMA, and UDMA were undetectable before removal but were found to be quantifiable after removal, with Bis-GMA and UDMA showing measurable levels. In conclusion, elevated BPA levels were found in patients treated with Invisalign™ and flowable resin composite attachments.

Azhagudurai N, Rajendran R, Aishwarya K et al (2025)[61] aimed to detect and quantify bisphenol A (BPA) leaching in salivary samples of patients undergoing clear aligner therapy (CAT) using four different commercially available sheets. Four types of clear aligners, namely Monoflex®, Erkodur®, Leone®, and Duran®, were provided to 20 volunteers, with five participants in each group (group A, B, C, and D, respectively). Salivary samples were collected at four different time points: immediately before aligner insertion (day 0) and then at day 1, day 5, and day 7. . The results showed an overall mean BPA leaching of 0.74 ± 0.33 ppm on day 1 (T1) across all groups, with no detectable leaching on days 5 (T5) and 7 (T7). Among the four aligners, the highest BPA leaching was observed in Duran (1.24 ppm) at day 1, followed by Monoflex (0.76 ppm), Erkodur (0.56 ppm), and Leone (0.43 ppm).In conclusion, BPA leaching occurred predominantly within the first 24 hours of aligner use, with no leaching detected in the later stages of the treatment. Duran exhibited the highest level of leaching and was considered the least safe, followed by

Monoflex, Erkodur, and Leone.

Peter E, Monisha J, Sylas VP (2025)[62] conducted this study, aimed to analyze the smoke composition generated from incinerating two common clear aligner materials, glycol-modified polyethylene terephthalate (PET-G) and polyurethane, which are often disposed of with general plastic waste and incinerated. The resultant smoke was collected and analyzed using gas chromatography-mass spectrometry (GC-MS) to study the in-depth composition of the smoke. The results identified a total of 20 compounds from each material, with Oxylene (21.06%) having the highest concentration in the PET-G sample and 1,4-dimethyl-1,3-cyclohexadiene (18.88%) in the polyurethane sample. The first peak in the PET-G sample corresponded to benzene, with a relative concentration of 5.18%. Four compounds were found in both samples: 1,4-dimethyl-1,3-cyclohexadiene, 1,3-cyclohexadiene (2,3-dimethyl-), 1-hydroxymethyl-4-methylenecyclohexane, and cyclohexanemethanol (4-methylene-). The study concluded that the incineration of PET-G produced benzene, a group 1 carcinogen, while polyurethane released tetrahydrofuran, a suspected carcinogen.

Materials And Method

MATERIALS AND METHOD

STUDY SITE

Department of Orthodontics and Dentofacial Orthopaedics, Santosh Dental College and Hospital, Ghaziabad, NCR, Delhi - 201009

Drug Standardisation Research Institute, Kamla Nehru Nagar, Ghaziabad - 201002

STUDY DESIGN

A Cross-Sectional Observational Study

STUDY TYPE

In - Vitro study

INCLUSION CRITERIA FOR SELECTION OF STUDY SUBJECTS

Thermoplastic aligner/retainer (retrieved/as-received) used in clinical or in-vitro research which can be thermoformed or pressed for aligner/ retainer use.

Materials tested:

1. Polyethylene (COPYPLAST®)
2. Polyethylene terephthalate-Glycol (DURAN®+)
3. Polycyclohexylenedimethylene terephthalate glycol-modified (IMPRELON® S)

EXCLUSION CRITERIA FOR SELECTION OF STUDY SUBJECTS

Raw thermoplastic materials/sheets used for aligners/retainers but not pressed and formed as aligners/retainers for clinical use.

SAMPLE SIZE CALCULATION

A total of 4 ethanol concentrations were used for analysis of 3 different materials in this in vitro study. Each specimen was tested in triplicate, resulting in 36 data points. This design was chosen based on the highly controlled nature of in vitro experimentation, which typically reduces biological variability and allows for smaller sample sizes while maintaining statistical relevance.

ARMAMENTARIUM

1. **Thermoplastic sheets**

- DURAN®+ - Polyethylenterephthalate-Glycol (PETG), Scheu Dental GmbH Am Burgberg Iserlohn Germany.
- COPYPLAST®- Polyethylene (PE), Scheu Dental GmbH Am Burgberg Iserlohn Germany.
- IMPRELON® S polycyclohexylenedimethylene terephthalate glycol-modified (PCTG), Scheu Dental GmbH Am Burgberg Iserlohn Germany. (Figure 1)

2. Thermoforming machine

- BIOSTAR® - Scheu Dental GmbH Am Burgberg Iserlohn Germany (Figure 2)

3. **3D printing resin -** D-Tech Accuprint model pro, D- tech dental technology. (Figure 3)

4. **3D printer -** Asiga Max 2 (DLP technology) (Figure 4)

5. **Gas Chromatography mass spectrometry unit -** Thermo Scientific TSQ 9610 triple quadrupole GC-MS/MS system.© 2006-2025 Thermo Fisher Scientific Inc (Figure 5)

6. **Neya 16 centrifuge** REMI Sales & Engineering Ltd. (Figure 6)

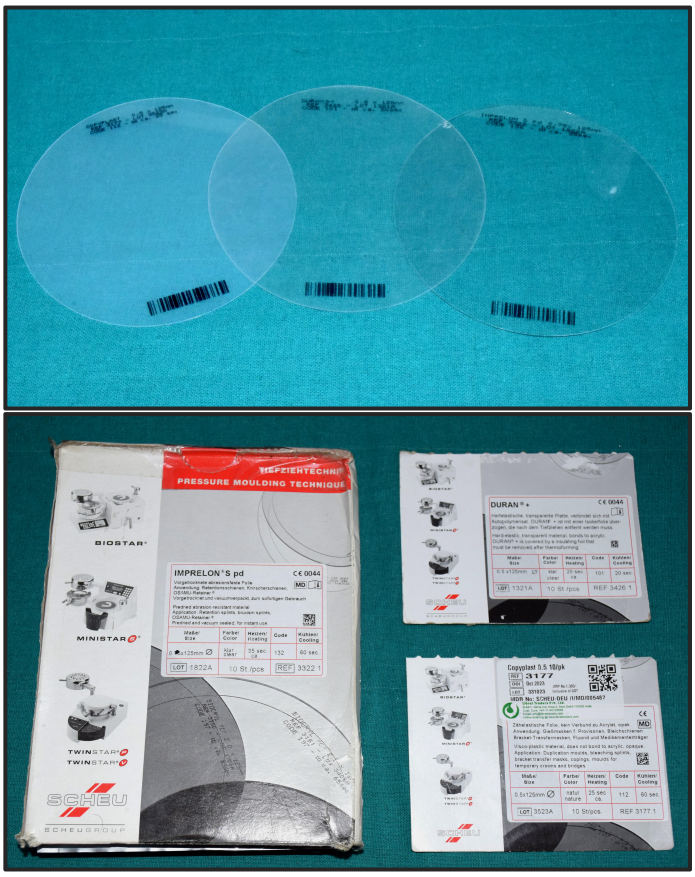


Figure 1. DURAN®+, COPYPLAST®, IMPRELON® S aligner sheets

Figure 2. BIOSTAR® - Scheu Dental GmbH Am Burgberg Iserlohn Germany

Figure 3. 3D printing resin D-Tech Accuprint model pro

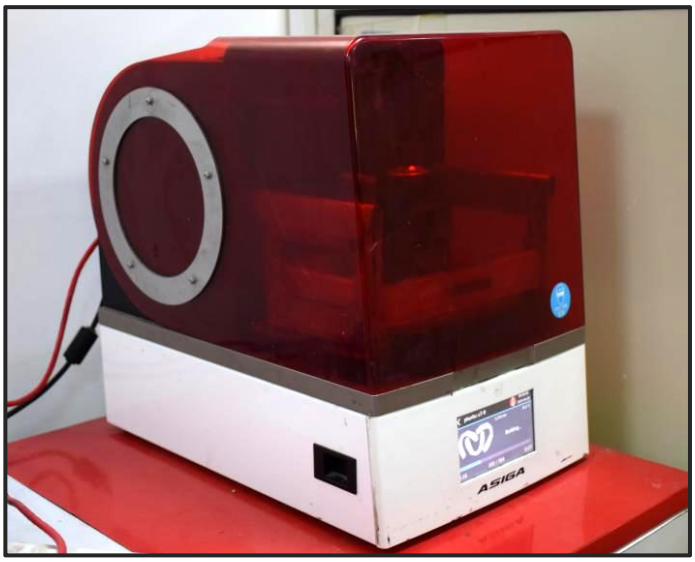


Figure 4. Asiga Max 2

Figure 5. Thermo Scientific TSQ 9610 triple quadrupole GC-MS/MS system

Figure 6. Neya 16 centrifuge

Figure 7. Thermoformed aligner sheets

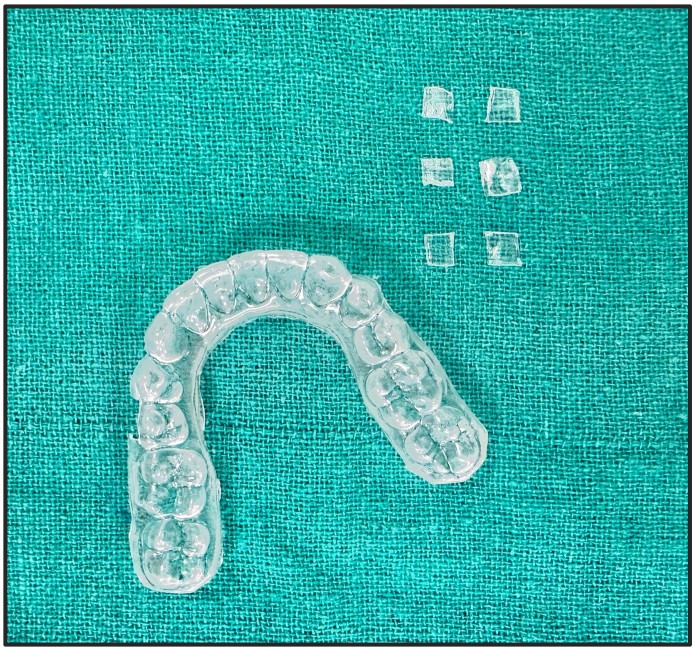


Figure 8. 5 X 5 mm aligner sheet cutouts

METHODOLOGY

Three sets of aligners trays of different materials namely Polyethylene, Polyethylene terephthalate-Glycol, Copolyester were obtained from the same manufacturer (Scheu Dental GmbH Am Burgberg Iserlohn Germany).

The sheets were thermoformed on identical printed models using a Biostar vaccum forming machine (Figure 5). Accordingly 5x5 mm squares of the sheet were cut from the anterior region of the cast of all 3 sheets.(Figure 8)

Each of them was immersed in separate glass sample vials containing alcoholic media to accelerate the degradation of the sample. Each material was soaked in four different solutions as follows: the first was absolute ethanol (100%), the second was 75% ethanol to 25% water, the third was 50% ethanol to water, the fourth was 25% ethanol to 75% water, and finally, 100% (Figure 9). The samples were incubated for two weeks at 37 ^{0}C and shaken for five minutes at 150 rpm every day to simulate the accelerated aging process.[14] To evaluate the time-dependent effects of leaching, samples were retrieved and analyzed at two intervals after 7 days and after 14 days of immersion.

The leached substances in the immersion medium were analyzed with a gas chromatography–mass spectrometry unit. A Thermofischer TSQ 9000 combined with a triple quadrupole mass spectrometer was used for GC-MS analysis. The chromatographic column was an TSQ-9000 5MS column (30 m × 0.25 mm × 0.25 μm film thickness), with high-purity helium as the gas carrier, at a flow rate of 1 mL/min.

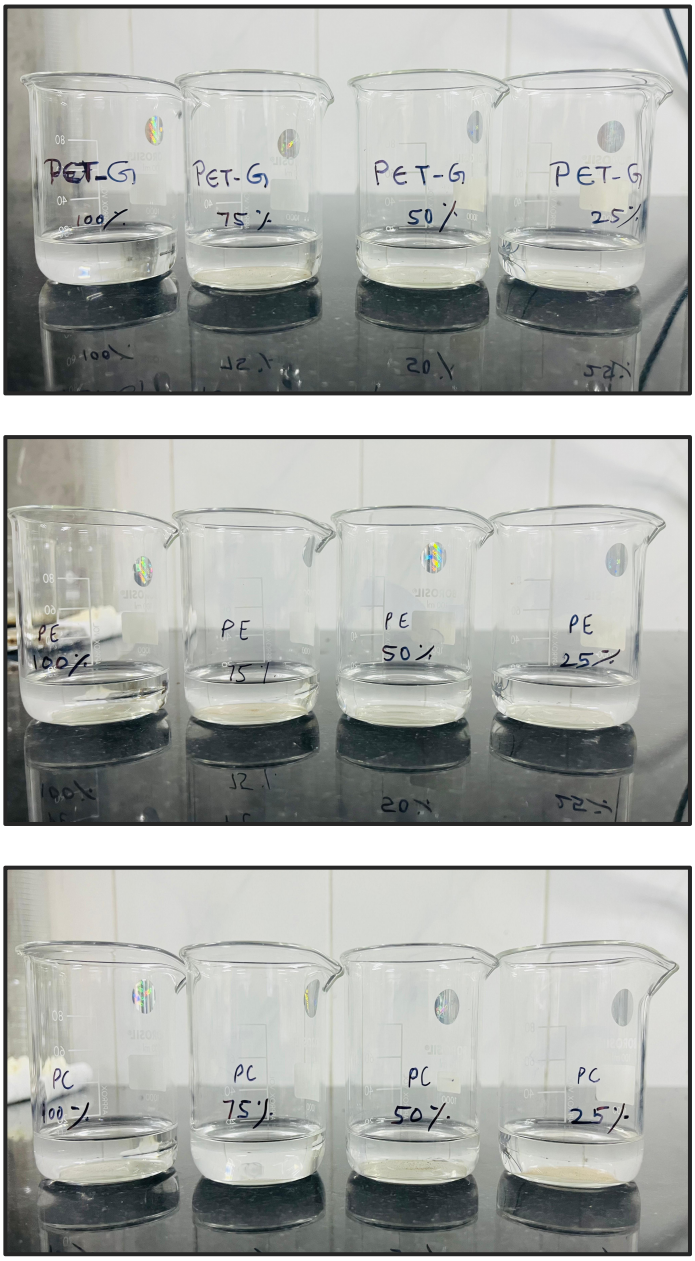


Figure 9. Material soaked in four different ethanol solutions 25%, 50%, 75%, 100%

The injector temperature was 260 ^{0}C, and it was equipped with a splitless injector at 20:1. The source temperature of MS was set at 300 ^{0}C, and the quad temperature was at 150 ^{0}C.

The oven temperature was initially 80 ^{0}C (held for 1.5 min), then it was increased to 250 ^{0}C at the rate of 10 ^{0}C min (held for 1 min), then it was further increased to 280 ^{0}C at the rate of 25 ^{0}C min (held for 1 min). The scan range was set at 50–300 mass ranges at 70 eV electron energy and a solvent delay of three minutes. Total runtime was estimated to be 26.5mins. Finally, unknown compounds were identified by comparing the spectra with those of the NIST 2017 (National Institute of Standard and Technology library).[63]

STATISTICAL ANALYSIS

Data analysis was performed using one-way Analysis of Variance (ANOVA) to compare the mean leaching levels of compounds from three different aligner materials Polyethylene (PE), Polyethylene Terephthalate Glycol (PET-G), and polycyclohexylenedimethylene terephthalate glycol-modified (PCTG) under varying ethanol concentrations (25%, 50%, 75%, and 100%) and two time intervals (7 and 14 days). Each group included four specimens, tested in triplicate, yielding 12 data points per group.

For each condition, the mean and standard deviation (SD) were calculated. ANOVA was used to assess whether differences in leaching levels between materials were statistically significant. A p-value less than 0.01 was considered significant. In conditions where no leaching was detected and all values were zero, statistical analysis was not applicable (F = NA, p = NA).

The data obtained was subjected to statistical analysis. The data so obtained was compiled systematically. A master table was prepared and the total data was subdivided and distributed meaningfully and presented as individual tables along with graphs.

Statistical procedures were carried out in 2 steps:

1. Data compilation and presentation
2. Statistical analysis

Statistical tests employed for the obtained data in our study were:

1. Mean: Individual observations were combined together & then divided by the number of observations to obtain the mean. The symbol S denotes the operation of combining or summarizing.

The individual observation is denoted by the sign X, number of observation denoted by n, & the mean by $\overline{X}$.

$$\overline{X} = \frac{\Sigma X}{\text{No. of observations (n)}}$$

2. Standard Deviation: It is denoted by the Greek letter s. If a sample is more than 30 then.

$$\sigma = \sqrt{\frac{\Sigma (X - \overline{X})^2}{n}}$$

When sample in less than 30 then,

$$\sigma = \sqrt{\frac{\Sigma (X - \overline{X})^2}{n-1}}$$

3. Analysis of Variance (ANOVA):

One way ANOVA was used to compare the mean and their components between the groups. It tests the significance of difference between the means of more than two groups. It compares group means by analyzing comparisons of variance estimates. Assumptions in one way ANOVA are-

subjects are sampled randomly, groups are independent, population variances are homogenous, population distribution is norm.

Numerically, one way ANOVA is a generalisation of the two sample t test. The F statistic compares the variability between the groups to the variability within the groups:

$$F = \frac{MST}{MSE}$$

$$MST = \frac{\sum_{i=1}^{k}(T_i^2/n_i) - G^2/n}{k-1}$$

$$MSE = \frac{\sum_{i=1}^{k}\sum_{j=1}^{n_i} Y_{ij}^2 - \sum_{i=1}^{k}(T_i^2/n_i)}{n-k}$$

where F is the variance ratio for the overall test, MST is the mean square due to treatments/groups (between groups), MSE is the mean square due to error (within groups, residual mean square), Yij is an observation, Ti is a group total, G is the grand total of all observations, ni is the number in group i and n is the total number of observations.

Assumptions:

- Random samples
- Normally distributed observations in each population
- Equal variance of observations in each population

All analyses were conducted under the assumption of normal distribution and equal variance, consistent with standard in vitro protocols. The data were compiled and analyzed using Statistical Package for the Social Sciences (SPSS) software version 26.0 (IBM Inc., Chicago, IL, USA). Descriptive statistics (means, standard deviations, and frequencies) were used to express all quantitative variables.

Results

RESULTS

Immersion of PE, PET-G and PCTG in different concentrations of ethanol for 7 days

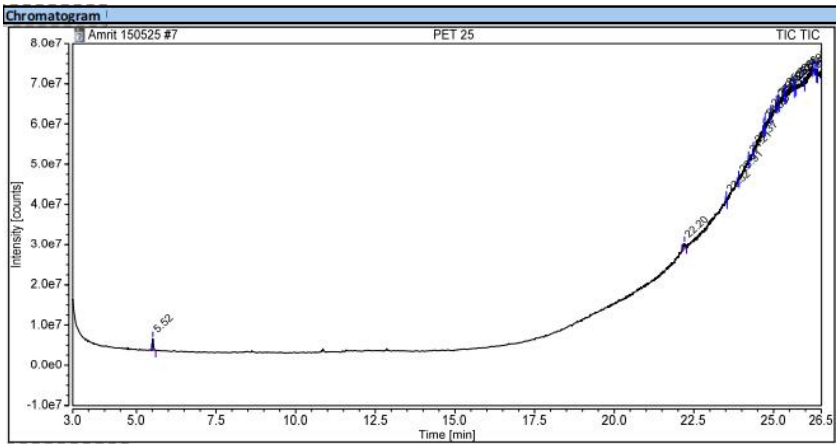
In the 7-day immersion group, trace ion signals were detected in some samples; however, the intensity levels were extremely low and inconsistent, falling close to the baseline instrument noise (Graphs 1, 2, 3, 4). These readings were not reproducible across replicates and did not correspond to identifiable compounds in the GC-MS library, suggesting that no meaningful leaching occurred during this short-term exposure period, and the signals were likely attributable to instrumental background noise.

Table 1. Comparison of mean leaching of compound on immersion of PE, PET-G and PCTG in 25% ethanol for 7 days

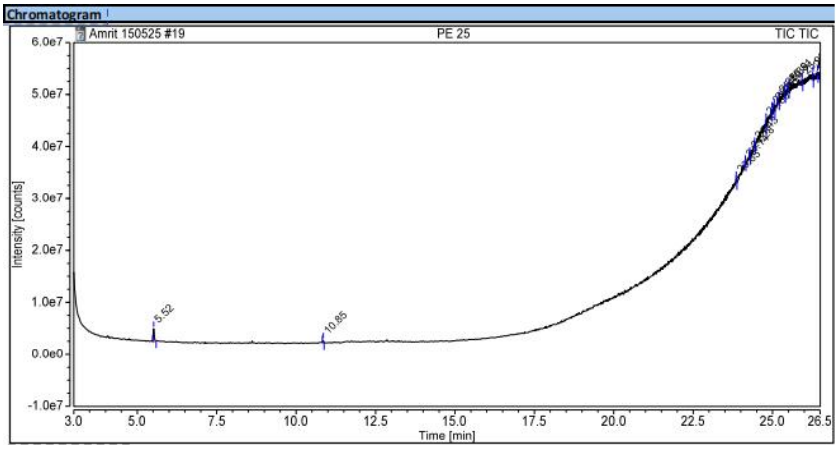
PE		**PET-G**		**PCTG**		**F value; p value**
Mean	SD	Mean	SD	Mean	SD	
0.000	0.000	0.000	0.000	0.000	0.000	NA

Table 1 reports no detectable leaching when the materials were immersed in 25% ethanol for 7 days. All means and standard deviations were 0.000 for the compound analyzed. Statistical testing was not applicable (F and p = NA).

Graph 1. Chromatogram of PET-G in 25% ethanol concentrations for 7 days



Graph 2. Chromatogram of PE in 25% ethanol concentrations for 7 days



Graph 3. Chromatogram of PCTG in 25%, ethanol concentrations for 7 days

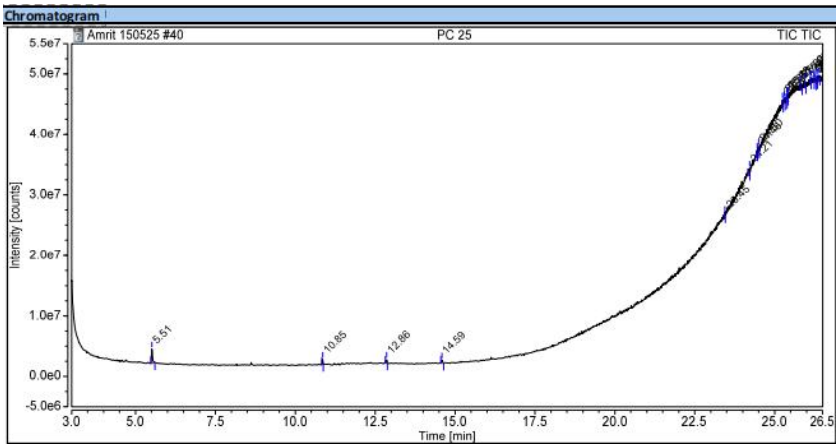
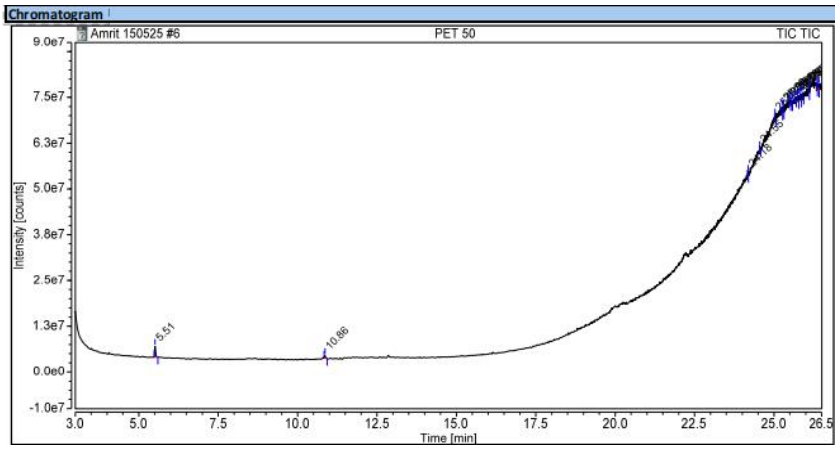


Table 2. Comparison of mean leaching of compound on immersion of PE, PET-G and PCTG in 50% ethanol for 7 days

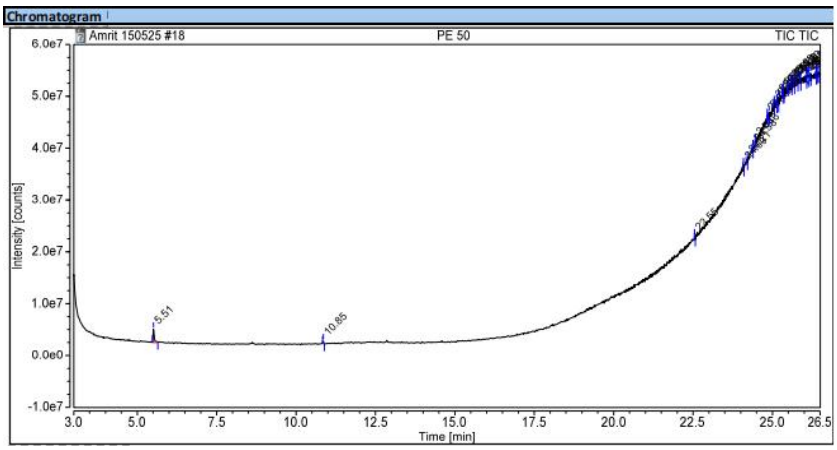
PE		PET-G		PCTG		F value; p value
Mean	SD	Mean	SD	Mean	SD	
0.000	0.000	0.000	0.000	0.000	0.000	NA

Table 2 also showed no leaching for any material in 50% ethanol after 7 days. All values remained at 0.000 with no standard deviation, leading to an F value and p value of NA.

Graph 4 - Chromatogram of PET-G in 50% ethanol concentrations for 7 days



Graph 5. Chromatogram of PE in 50% ethanol concentrations for 7 days



Graph 6. Chromatogram of PCTG in 50% ethanol concentrations for 7 days

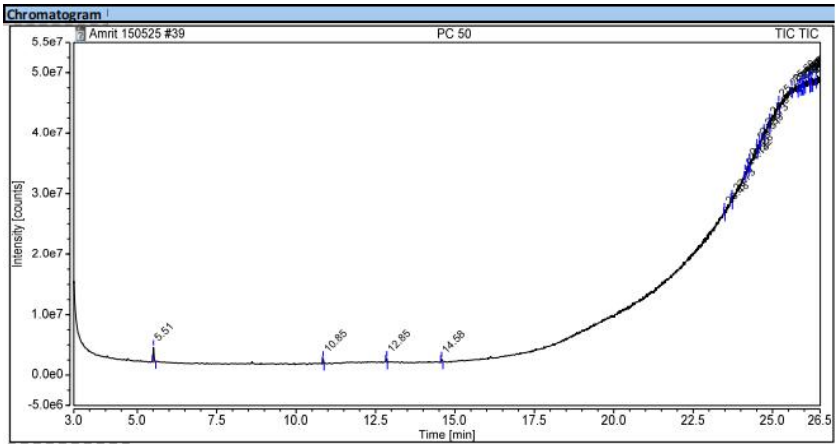
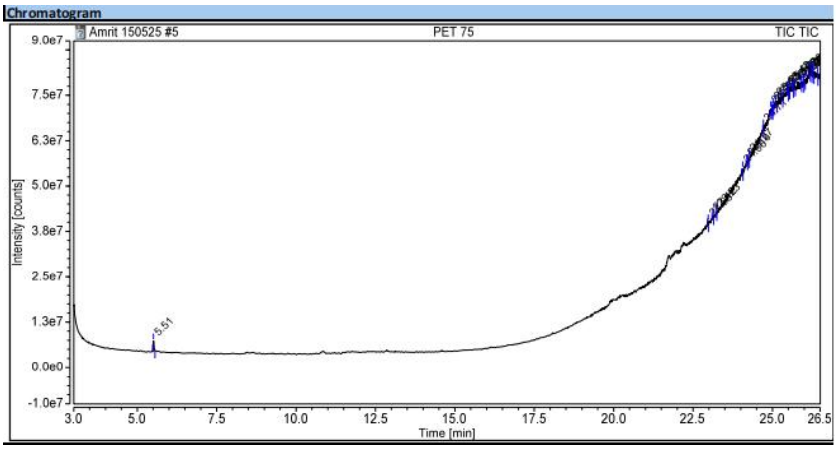


Table 3. Comparison of mean leaching of compound on immersion of PE, PET-G and PCTG in 75% ethanol for 7 days

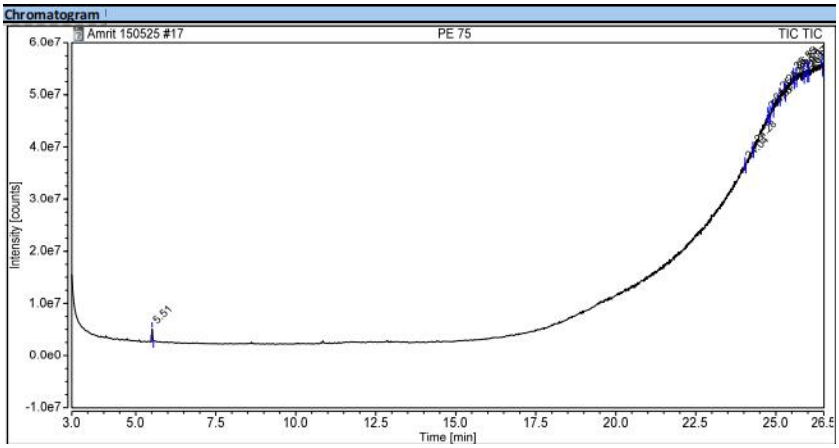
PE		PET-G		PCTG		F value; p value
Mean	SD	Mean	SD	Mean	SD	
0.501	0.001	0.506	0.007	0.501	0.001	1.53;.295

Table 3 includes data from 75% ethanol exposure for 7 days. PE and PCTG had the same mean leaching level (0.501), with SDs of 0.001, while PET-G showed a slightly higher mean of 0.506 (SD 0.007). The F value was 1.53 with a p value of 0.295, indicating no statistically significant difference.

Graph 7. Chromatogram of PET-G in 75% ethanol concentrations for 7 days



Graph 8. Chromatogram of PE in 75% ethanol concentrations for 7 days



Graph 9. Chromatogram of PCTG in 75% ethanol concentrations for 7 days

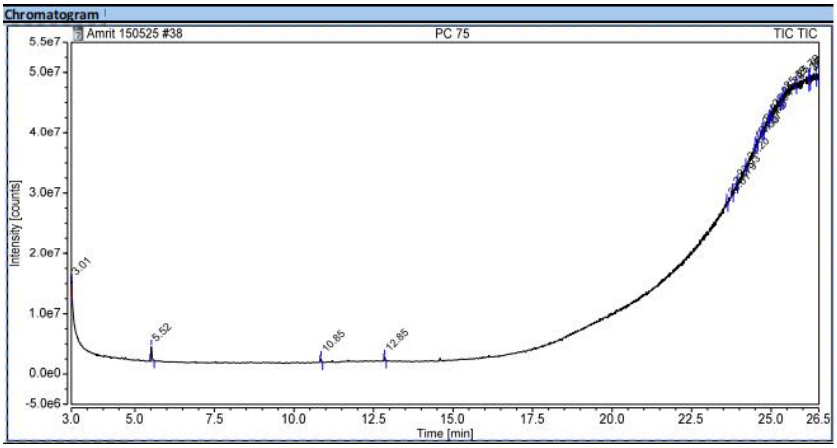
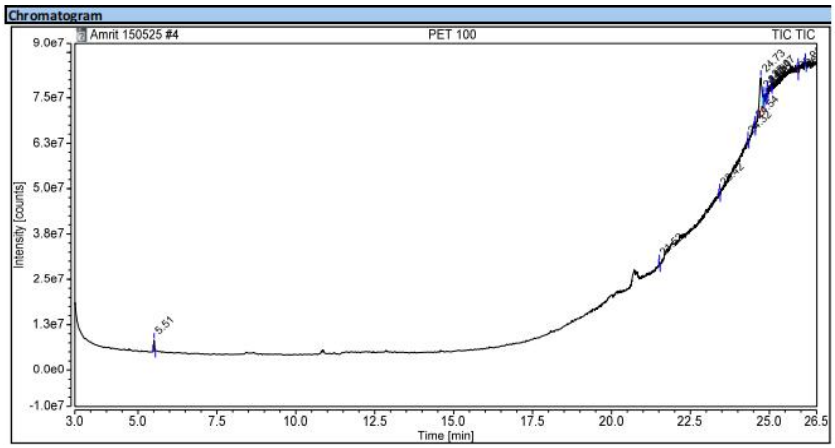


Table 4. Comparison of mean leaching of compound on immersion of PE, PET-G and PCTG in 100% ethanol for 7 days

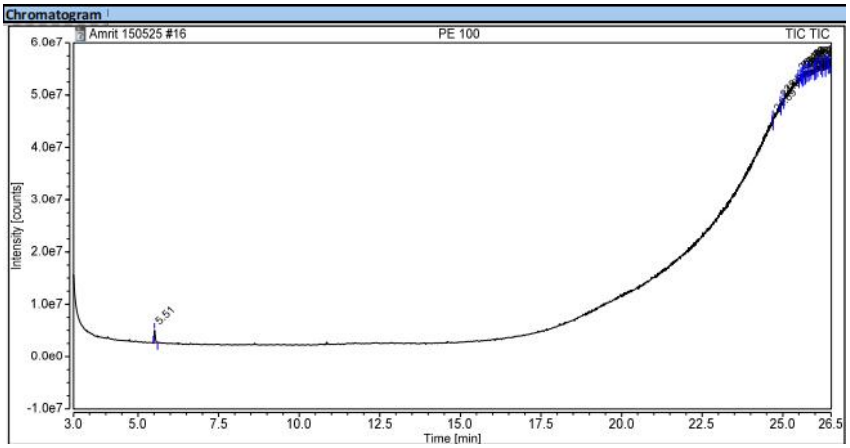
PE		PET-G		PCTG		F value; p value
Mean	SD	Mean	SD	Mean	SD	
0.500	0.001	0.510	0.003	0.509	0.003	30.0;<.01*

Table 4 presents leaching levels after 7 days in 100% ethanol. PE leached 0.500 (SD 0.001), PET-G 0.510 (SD 0.003), and PCTG 0.509 (SD 0.003). ANOVA showed an F value of 30.0 with $p < 0.01$, indicating a statistically significant difference among the materials.

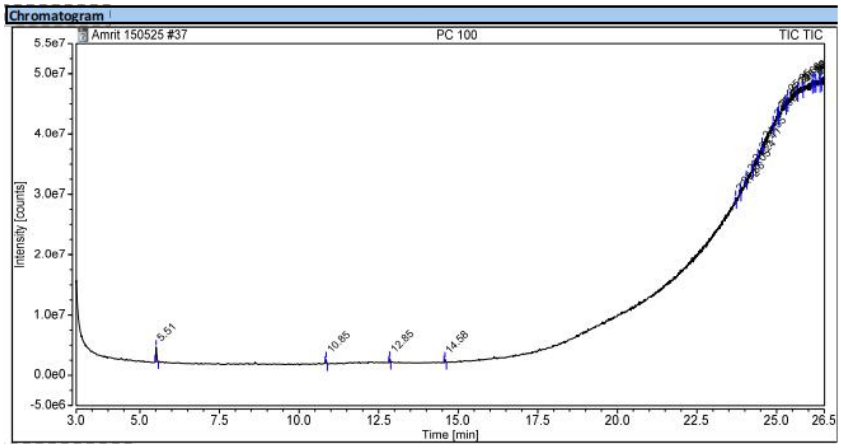
Graph 10. Chromatogram of PET-G in 100% ethanol concentrations for 7 days



Graph 11. Chromatogram of PE in 100% ethanol concentrations for 7 days



Graph 11. Chromatogram of PE in 100% ethanol concentrations for 7 days



Immersion of PE, PET-G and PCTG in different concentrations of ethanol for 14 days

In contrast, the 14-day immersion group demonstrated distinct and consistent leaching of two identifiable compounds across all 3 aligner materials.

Compound 1 - Hexadecanoic acid, methyl ester

Compound 2 - Heptadecanoic acid, 16-methyl-, methyl ester

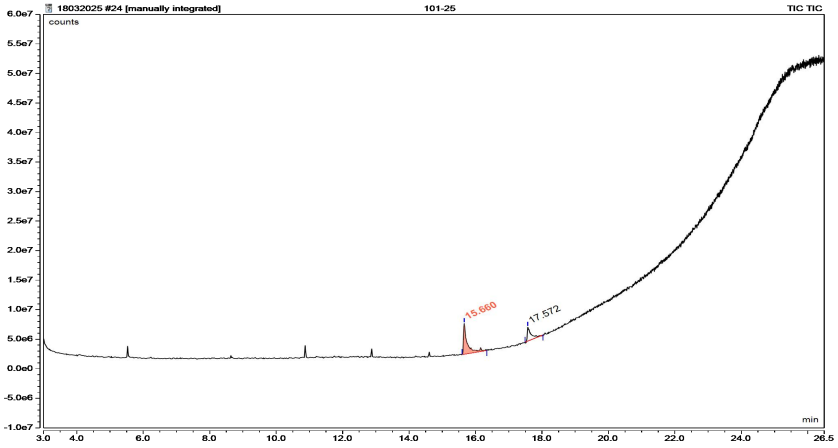
No compound leaching was detected in the 25% ethanol solution after 14 days; however measurable release of compounds was observed in the 50%, 75%, and 100% ethanol solutions.

Table 5. Comparison of mean leaching of compounds(1&2) when three different materials are immersed in 25% ethanol for 14 days

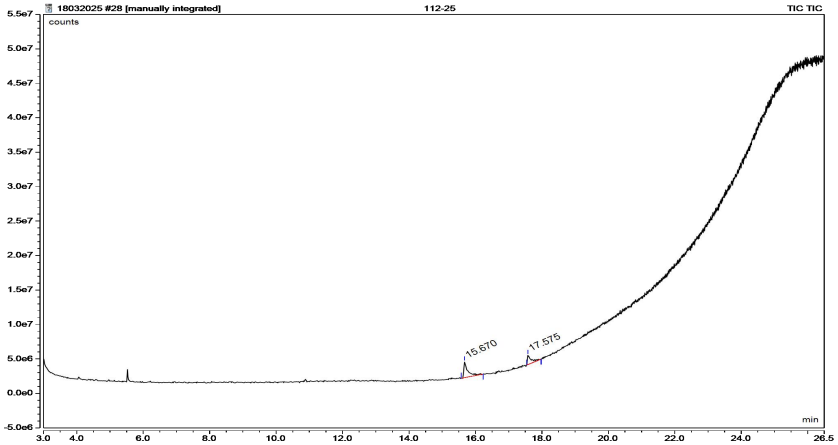
	PE		PET-G		PCTG		F value; p value
	Mean	SD	Mean	SD	Mean	SD	
Compound 1	0.000	0.000	0.000	0.000	0.000	0.000	NA
Compound 2	0.000	0.000	0.000	0.000	0.000	0.000	NA

Table 5 presents the comparison of mean leaching levels of Compounds 1 and 2 when three materials (PE, PET-G, and PCTG) were immersed in 25% ethanol for 14 days. No leaching was detected for either compound across all materials, resulting in a mean and standard deviation of 0.000 for each. As there was no variability, statistical analysis was not applicable (F value and p value = NA).

Graph 13. Chromatogram of PET-G in 25% ethanol concentrations for 14 days



Graph 14. Chromatogram of PE in 25% ethanol concentrations for 14 days



Graph 15. Chromatogram of PCTG in 25% ethanol concentrations for 14 days

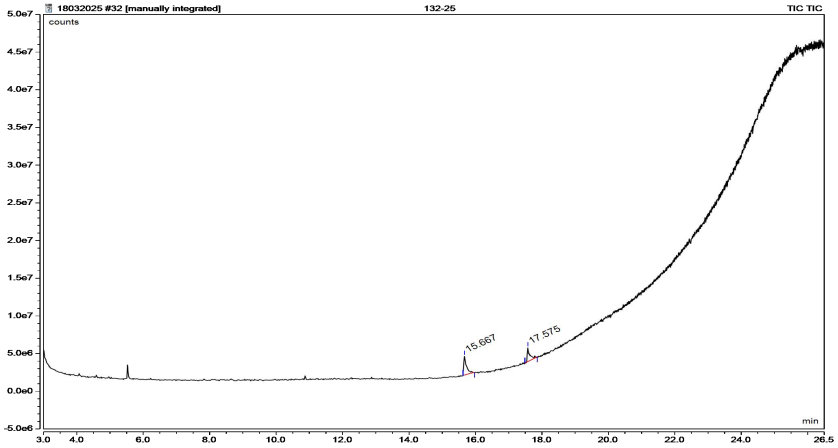
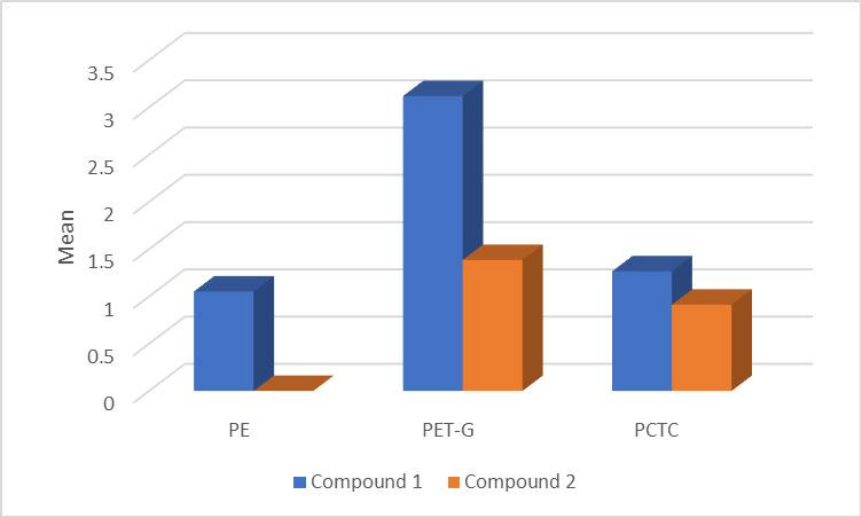


Table 6. Comparison of mean leaching of compound on immersion of PE, PET-G and PCTG in 50% ethanol for 14 days.

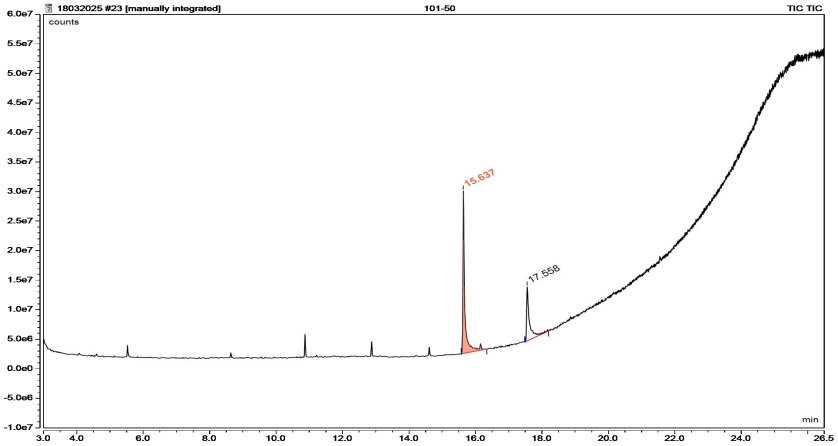
	PE		PET-G		PCTG		F value; p value
	Mean	SD	Mean	SD	Mean	SD	
Compound 1	1.052	0.043	3.119	0.068	1.265	0.070	1022.84;<.01*
Compound 2	0.000	0.000	1.386	0.028	0.912	0.029	2748..38;<.01*

Table 6 shows the leaching of Compounds 1 and 2 in 50% ethanol over 14 days. For Compound 1, PE showed a mean of 1.052 (SD 0.043), PET-G 3.119 (SD 0.068), and PCTG 1.265 (SD 0.070). The ANOVA yielded an F value of 1022.84 with a p value <.01, indicating a significant difference. Compound 2 showed no leaching from PE (0.000), but PET-G and PCTG had means of 1.386 (SD 0.028) and 0.912 (SD 0.029), respectively, with an F value of 2748.38 and p <.01, also statistically significant.

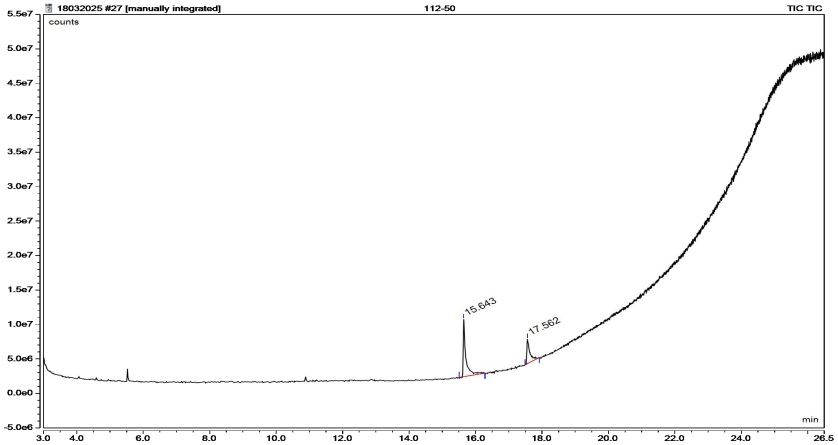
Graph 16. Comparison of mean leaching of compound on immersion of PE, PET-G and PCTG in 50% ethanol for 14 days



Graph 17. Chromatogram of PET-G in 50% ethanol concentrations for 14 days



Graph 18. Chromatogram of PE in 50% ethanol concentrations for 14 days



Graph 19. Chromatogram of PCTG in 50% ethanol concentrations for 14 days

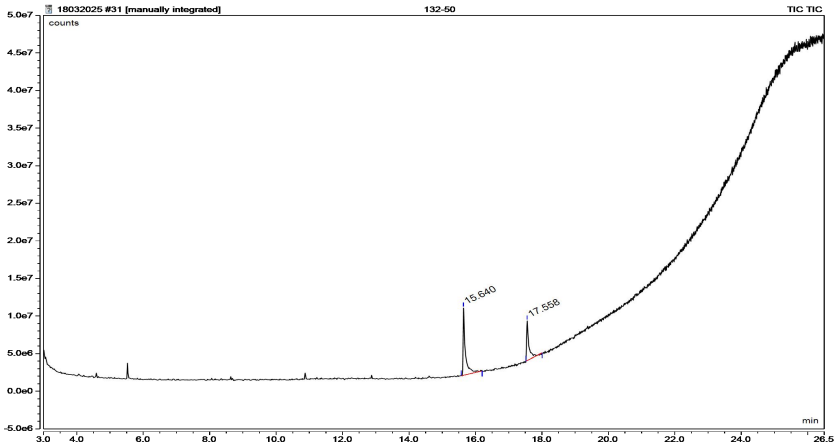
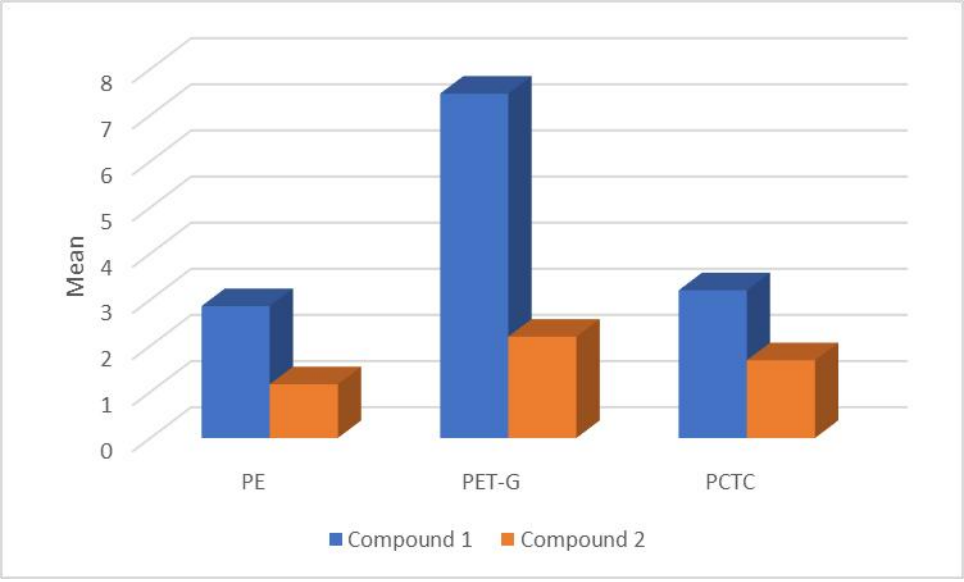


Table 7. Comparison of mean leaching of compound on immersion of PE, PET-G and PCTG in75% ethanol for 14 days.

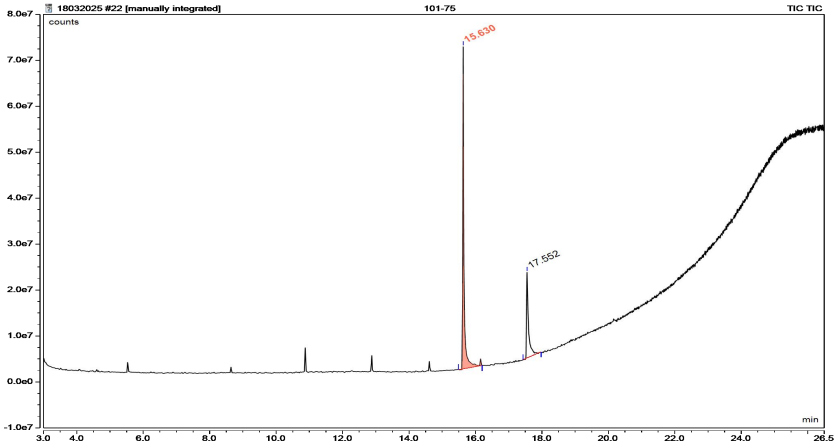
	PE		PET-G		PCTG		F value; p value
	Mean	SD	Mean	SD	Mean	SD	
Compound 1	2.860	0.097	7.467	0.080	3.203	0.064	2978.43;<.01*
Compound 2	1.164	0.053	2.197	0.044	1.685	0.063	275.53;<.01*

Table 7 summarizes results in 75% ethanol over 14 days. Compound 1 leaching averaged 2.860 (SD 0.097) for PE, 7.467 (SD 0.080) for PET-G, and 3.203 (SD 0.064) for PCTC. The F value was 2978.43 with p <.01, signifying a strong statistical difference. For Compound 2, PE had 1.164 (SD 0.053), PET-G 2.197 (SD 0.044), and PCTC 1.685 (SD 0.063), with F = 275.53 and p <.01, indicating significant variation among materials.

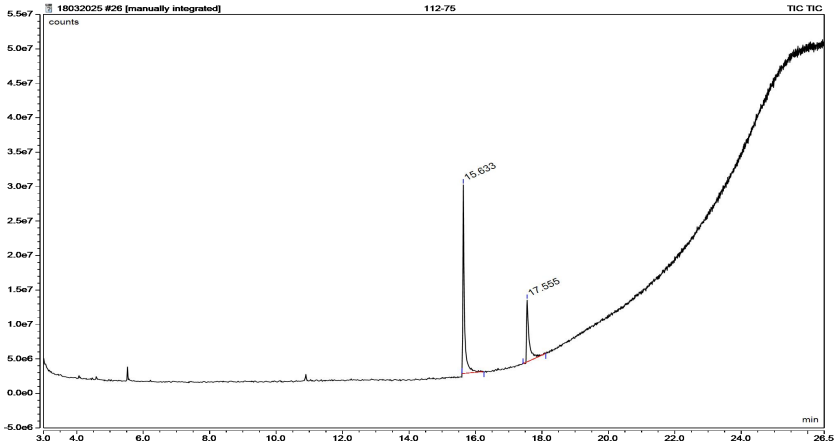
Graph 20. Comparison of mean leaching of compound on immersion of PE, PET-G and PCTG in 75% ethanol for 14 days



Graph 21. Chromatogram of PET-G in 75% ethanol concentrations for 14 days



Graph 22. Chromatogram of PE in 75% ethanol concentrations for 14 days



Graph 23. Chromatogram of PCTG in 75% ethanol concentrations for 14 days

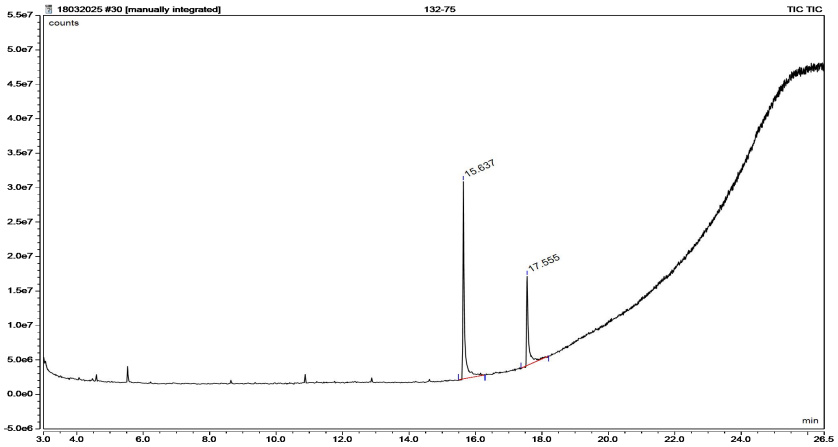
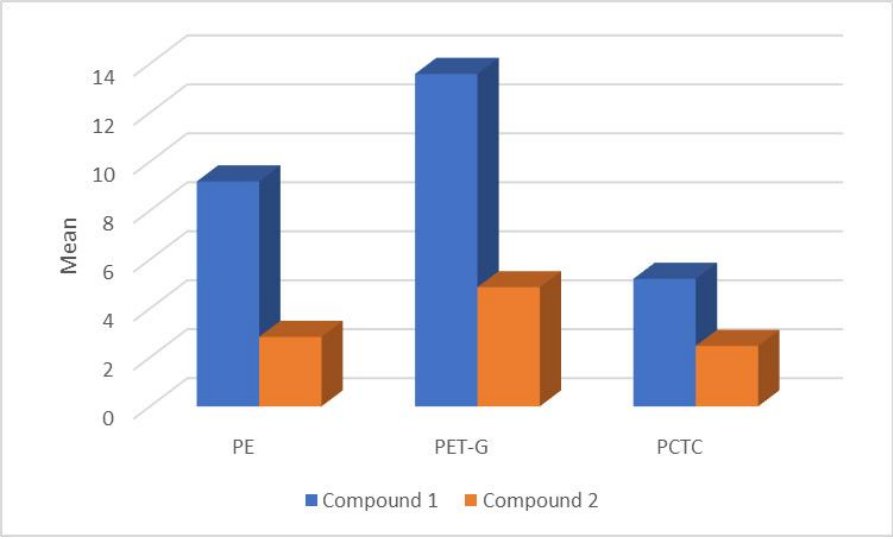


Table 8. Comparison of mean leaching of compound on immersion of PE, PET-G and PCTG in 100% ethanol for 14 days.

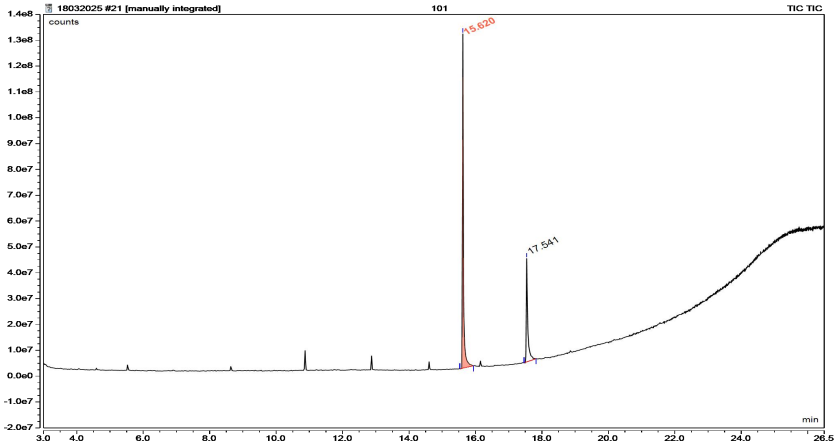
	PE		PET-G		PCTG		F value; p value
	Mean	SD	Mean	SD	Mean	SD	
Compound 1	9.190	0.074	13.583	0.123	5.212	0.102	5084.97;<.01*
Compound 2	2.840	0.095	4.867	0.050	2.470	0.046	1098.66;<.01*

Table 8 covers 100% ethanol immersion for 14 days. Compound 1 leached at 9.190 (SD 0.074) from PE, 13.583 (SD 0.123) from PET-G, and 5.212 (SD 0.102) from PCTG. The F value was 5084.97 with p <.01. For Compound 2, PE recorded 2.840 (SD 0.095), PET-G 4.867 (SD 0.050), and PCTG 2.470 (SD 0.046), with an F value of 1098.66 and p <.01. Both compounds exhibited statistically significant differences in leaching.

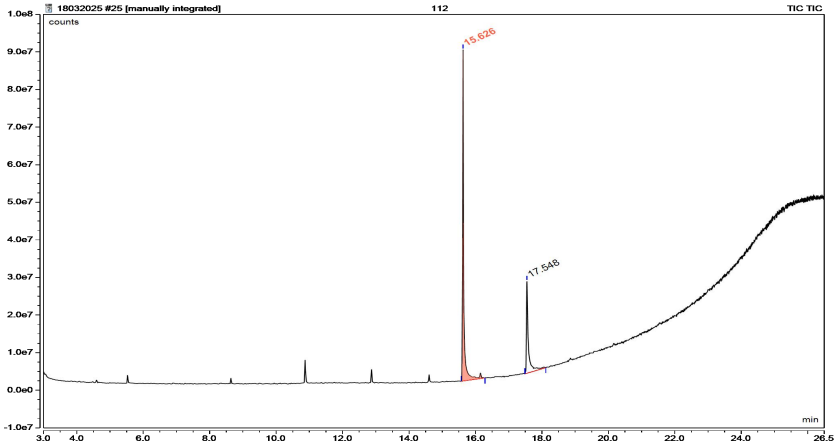
Graph 24. Comparison of mean leaching of compound on immersion of PE, PET-G and PCTG in 100% ethanol for 14 days



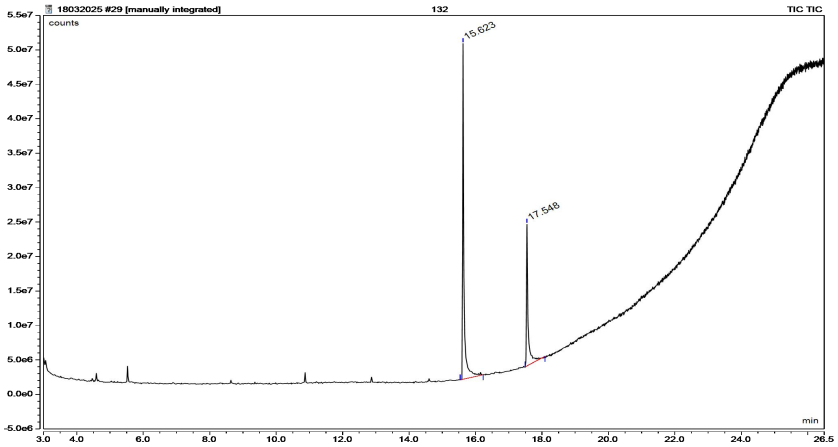
Graph 25. Chromatogram of PET-G in 100% ethanol concentrations for 14 days



Graph 26. Chromatogram of PE in 100% ethanol concentrations for 14 days



Graph 27. Chromatogram of PCTG in 100% ethanol concentrations for 14 day



Discussion

DISCUSSION

The assessment of chemical leaching from orthodontic materials is a critical component in evaluating their biocompatibility and long-term safety. Clear aligners, being in prolonged and intimate contact with the oral mucosa, are constantly exposed to variable conditions such as heat, salivary enzymes, dietary acids, and mechanical stress.

The present in vitro study investigated the leaching behavior of three commonly used clear aligner materials Polyethylene (Copyplast), Polyethylene Terephthalate Glycol (DURAN®+), and Polycyclohexylenedimethylene terephthalate glycol-modified (IMPRELON® S) taken from a common manufacturer Scheu Dental GmbH Am Burgberg Iserlohn Germany. following immersion in varying concentrations of ethanol (25%, 50%, 75%, 100%). The goal was to assess their chemical stability and potential for releasing harmful compounds when subjected to simulated accelerated ageing, which is responsible for increased solubility of these materials described by Wu and McKinney (1982).[14]

As shown in Table 1, Graph 1, immersion in 25% ethanol resulted in no detectable leaching for any of the materials, with mean and standard deviation values recorded as 0.000. Similarly, Table 2 reported no measurable leaching at 50% ethanol after 7 days, suggesting that these lower ethanol concentrations were insufficient to cause significant degradation or ion release within the tested timeframe.

At higher concentrations, a slight increase in ion concentration was observed. As seen in Table 3, immersion in 75% ethanol for 7 days resulted in detectable ion levels in all three materials, with PET-G showing a marginally higher mean value. However, the F value (1.53) and p value (0.295) indicate no statistically significant differences among

the groups. Additionally, the detected concentrations were close to the instrument's limit of detection, suggesting the possibility of instrument noise rather than true compound identification.

In contrast, Table 4 demonstrates that immersion in 100% ethanol for 7 days resulted in statistically significant leaching across the materials ($F = 30.0$; $p < 0.01$), with PET-G and PCTG showing slightly higher ion release compared to PE. These results support the notion that elevated ethanol concentrations may serve as accelerants in simulating long-term intraoral degradation and identifying leachates. However, the absence of reliable compound matching through GC-MS in the 7-day groups suggests that compound concentrations remained low or below identification thresholds.

The 14-day immersion results revealed measurable and statistically significant compound leaching from all three aligner materials PE, PET-G, and PCTG when subjected to ethanol concentrations of 50%, 75%, and 100%.

In contrast, no detectable leaching of the two target compounds, Hexadecanoic acid, methyl ester and Heptadecanoic acid, 16-methyl-, methyl ester, was observed at 25% ethanol, as confirmed in Table 5, indicating that lower ethanol concentrations are insufficient to cause meaningful degradation or ion release under the tested conditions.

At 50% ethanol, Table 6 and Graph 16 showed that PET-G exhibited the highest leaching for both compounds, followed by PCTG, with PE showing the least. Compound 2 was not detected in PE, suggesting material-specific resistance at moderate solvent strength.

Leaching intensified at 75% ethanol (Table 7 and Graph 20), with significant release of both compounds across all three materials. PET-G consistently showed the highest

ion concentrations, reaffirming its greater susceptibility to ethanol-induced degradation, while PE demonstrated the lowest levels. The results suggest increasing ethanol concentration enhances compound dissolution and material breakdown.

Finally, at 100% ethanol (Table 8 and Graph 24), the most substantial leaching occurred, again with PET-G showing the greatest release, followed by PE and then PCTG. The statistically significant F and p values across all tests confirm material-dependent differences in degradation behavior.

Collectively, these findings emphasize that both compound detection and ion concentrations are ethanol concentration-dependent, with PET-G showing the highest degradation potential, particularly in stronger solvents. These results reinforce the utility of ethanol-based immersion as an accelerated aging model to evaluate bio-compatibility and chemical stability of aligner materials.

Two compounds were consistently detected in the 14-day group: Hexadecanoic acid, methyl ester (commonly known as methyl palmitate) and Heptadecanoic acid, 16-methyl-, methyl ester (commonly known as methyl isostearate). These compounds are typically derived from the breakdown of lipophilic polymeric components or additives used in plastics and resins.[65]

Hexadecanoic acid, methyl ester is a fatty acid methyl ester (FAME) commonly found in natural fats and oils and is often used as a plasticizer, lubricant, or intermediate in bio-diesel production.[65] According to its Material Safety Data Sheet (MSDS), it is generally regarded as non-toxic and non-carcinogenic, though prolonged exposure may cause mild irritation to the skin and eyes.[66]

Based on prior studies, Hexadecanoic acid, methyl ester has been widely reported to possess antioxidant, antimicrobial, antifungal, nematicidal, and hypocholesterolemic

properties, as well as activity against 5-alpha reductase, making it a compound of biological interest beyond the scope of material science. Its ability to act against bacterial and fungal organisms has been established in both plant-derived extracts and synthetic applications.[67]

Heptadecanoic acid, 16-methyl-, methyl ester,a branched-chain fatty acid methyl ester is recognized in industrial applications for its chemical stability and low toxicity profile. Supporting evidence from in vivo studies indicates that, this compound, referred to in some literature as HDA, exhibits no observable toxicity in healthy animal models. In a recent animal-based study, different concentrations of HDA (50 mg/mL, 100 mg/mL, and 150 mg/mL) were administered to evaluate its systemic toxicity. The group treated with 100 mg/mL HDA demonstrated 100% survival, strongly suggesting that the compound is biologically safe and well-tolerated at substantial concentrations. These findings validate the non-toxic nature of HDA in physiological systems and support its presence in aligner leachates as non-hazardous, especially considering the minimal quantities detected under accelerated aging conditions in vitro.[68]

GC-MS confirmed no residual release of monomers though the polar storage conidtions ethanol/water solutions were much more aggressive relative to oral environement. The minimal ion detection observed in the 7-day group was most likely due to instrumental background noise, as the signals were weak, inconsistent, and non-reproducible across replicates. This finding is in line with the understanding that short-term exposure may not sufficiently degrade these thermoplastic materials to allow compound release, especially in the absence of enzymatic or mechanical stresses found in vivo.

The findings of minimal leaching in the 7 and 14 days immersion groups in the present study are in agreement with previous research that has reported similarly low levels or absence of leachate release from clear aligner materials during short-term exposure. Schuster et al. in 2004 conducted an in vitro and in vivo analysis of aged and retrieved Invisalign® appliances and reported negligible monomer release, reinforcing the idea that these materials are chemically stable during initial wear periods.The results of this study, align with earlier investigations that have documented the chemical stability of clear aligner materials under simulated or clinical wear conditions.[8] Gracco et al. in 2009 reported that, Invisalign appliances retrieved after 14 days of intraoral use exhibited morphological changes such as micro-cracks, abrasions, delamination, and loss of transparency, but importantly, they found no evidence of monomer or by-product release when the aligners were subsequently suspended in artificial saliva.[8] This suggests that despite superficial and structural wear, the core material maintains its chemical inertness during this initial period of use.

A study done by Alhendi A et. al evaluating the chemical release from Invisalign®, Eon®, SureSmile®, and Clarity® aligners reported the detection of eleven different chemical compounds using GC-MS, with benzene, 1,3-bis(1,1-dimethylethyl) being the primary compound leached at 100% and 75% ethanol concentrations. No leaching was observed below 50% ethanol, suggesting a clear threshold effect for solvent-induced degradation. In contrast, the present study, which employed a similar ethanol gradient and 7 and 14 day immersion periods, detected only two compounds Hexadecanoic acid, methyl ester and Heptadecanoic acid, 16-methyl-, methyl ester and only after 14 days of immersion in higher ethanol concentrations.

Unlike the aforementioned study, which reported benzene-based compound release across multiple systems, no benzene or its derivatives were detected in our analysis.

Among the materials tested, PET-G demonstrated the highest leaching potential, followed by Polyethylene and copolyester. The increased leaching potential observed in PET-G may be attributed to its more complex polymeric structure or greater affinity to ethanol-induced degradation.

The stability of PET-G and its modified forms has emerged as a focal point in the evaluation of clear aligner materials due to their widespread use and favorable optical and mechanical properties. As observed in the present study, PET-G aligners demonstrated minimal chemical leaching at 7 days, with only mild compound release under high ethanol concentrations after 14 days. This suggests good short-term chemical stability, a finding that aligns with recent investigations assessing the physical and mechanical resilience of PET-G under simulated intraoral conditions.

Conventional PET-G, while mechanically robust, exhibits limitations such as reduced elastic recovery and progressive water absorption, which can lead to stress relaxation and reduced force delivery over time.[48] To address these concerns, modified PET-G variants have been developed by incorporating co-monomers like cyclohexane dimethanol (CHDM) or proprietary additives that enhance flexibility, toughness, and resistance to environmental stressors. These modified forms namely Polycyclohexylenedimethylene Terephthalate Glycol-Modified, demonstrate greater dimensional stability, improved fatigue resistance, and better adaptability to intraoral forces, making them more suited for long-term use in dynamic oral environments.[68] Furthermore, modified PET-G has shown a lower chemical leaching potential under controlled conditions than the conventional PET-G.

Stefano Martina et. al in 2019 conducted an in vitro study assessing the cytotoxicity of commonly used aligner materials—including SmartTrack and PETG—on human gingival fibroblasts (HGFs) found that, all tested materials exhibited slight cytotoxic effects after 14 days, which is the typical wear period for a single aligner. Notably, the study observed that thermoformed materials showed increased cytotoxicity compared to their non-thermoformed counterparts, suggesting that the thermoforming process may enhance the release of monomers, particularly in PETG-based materials, due to heat-induced degradation. However, the authors emphasized that the detected cytotoxicity was clinically negligible.[47]

Despite the controlled nature of in vitro immersion testing, it must be emphasized that such conditions may not fully capture the true degradation potential of aligner materials under intraoral conditions. In the oral cavity, aligners are continuously subjected to a wide range of mechanical and chemical challenges, including abrasion from mastication, attrition due to acidic beverages, and enzymatic activity in saliva. These dynamic factors, particularly at occlusal contact points like cusp tips, can cause surface wear, particle detachment, and gradual breakdown of the material.[8]

One important limitation of the present study stems from the reliance on GC-MS for compound identification, which inherently depends on the availability and accuracy of spectral libraries such as the NIST mass spectral library. While these databases are valuable tools for identifying unknown substances, including dental-related compounds, their spectra are primarily generated under standardized experimental conditions using "hard" ionization techniques. This can result in discrepancies when matching spectra from experimental GC-MS data due to variations in retention properties, ionization methods, or co-eluting compounds.

Furthermore, although mass spectral libraries increase confidence in substance identification, they should not be solely relied upon. As highlighted in the European Union regulation 2002/657/EC, co-eluting substances may compromise the fragmentation spectra, potentially leading to misidentification. To ensure accuracy, it is recommended that such qualitative analyses be supported by the use of calibration standards and internal controls. Additionally, library-based identification lacks sensitivity to specific experimental conditions, which may affect fragmentation patterns.

Therefore, while the compounds detected in the study were tentatively identified through spectral library comparison, the absence of reference standards and calibration solutions limits the confirmatory strength of these findings. Future studies should consider incorporating CAS registry-verified standards and internal validation protocols to strengthen the reliability of compound identification and quantification.[70]

Therefore, while in vitro methods provide a foundational understanding, retrieval analyses and in vivo studies remain essential for accurately characterizing the complex degradation patterns and biological consequences of aligner use in real-world settings.

Conclusion

CONCLUSION

- No significant chemical leaching was detected from Polyethylene (PE), Polyethylene Terephthalate Glycol (PET-G), or Polycyclohexylenedimethylene Terephthalate Glycol-Modified (PCTG) aligner materials after 7 days of immersion in ethanol solutions (25%, 50%, 75%, and 100%), with detected signals likely attributable to instrumental noise.
- After 14 days of immersion, measurable leaching occurred in 50%, 75%, and 100% ethanol solutions, indicating that prolonged exposure to higher ethanol concentrations induces material degradation.
- Two compounds, Hexadecanoic acid, methyl ester (methyl palmitate) and Heptadecanoic acid, 16-methyl-, methyl ester (methyl isostearate), were consistently detected in the 14-day immersion group across all three materials at higher ethanol concentrations.
- PET-G exhibited the highest leaching levels for both compounds, followed by PE and then PCTG, suggesting PET-G is more susceptible to ethanol-induced degradation.
- No detectable leaching of the identified compounds was observed in 25% ethanol after 14 days, indicating a threshold effect where lower ethanol concentrations are insufficient to cause significant degradation.
- The detected compounds are fatty acid methyl esters, generally regarded as non-toxic with low acute toxicity profiles, and some literature suggests they may have beneficial properties like antioxidant and antimicrobial effects.

Summary

SUMMARY

This in-vitro study explores the chemical stability and leaching potential of three widely used clear aligner materials Polyethylene (PE), Polyethylene Terephthalate Glycol-modified (PET-G), and Polycyclohexylenedimethylene Terephthalate Glycol-modified (PCTG). With the increasing popularity of clear aligner therapy in orthodontics, understanding the biocompatibility of these materials has become essential. The concern lies in the potential release of residual monomers or degradation byproducts, especially under prolonged intraoral exposure. While aligners are typically worn for extended durations, this study sought to simulate and accelerate their aging process under controlled laboratory conditions.

To achieve this, a total of 36 samples were prepared, comprising four specimens per material group, each tested in triplicate. The samples were immersed in four different ethanol-based solutions 25%, 50%, 75%, and 100% at 37°C for two durations: 7 days and 14 days. This ethanol-based immersion is commonly used in polymer testing to replicate oral conditions and assess potential material degradation. Following immersion, leachate analysis was performed using Gas Chromatography-Mass Spectrometry (GC-MS), a sensitive technique for detecting organic compounds at trace levels.

Results showed that in the 7-day immersion group, no significant leaching was detected for any of the materials across all ethanol concentrations. Minor ion detection was observed but considered background noise. However, in the 14-day immersion group, two compounds Hexadecanoic acid, methyl ester (methyl palmitate) and Heptadecanoic acid, 16-methyl-, methyl ester were consistently identified at higher ethanol concentrations. These compounds are recognized byproducts of

polymer degradation, often linked to lipophilic additives, and are reported to have low acute toxicity. PET-G exhibited the highest leaching among the materials, followed by PCTG and then PE.

The data demonstrated that longer immersion times and higher ethanol concentrations increased the likelihood of leaching, although the detected compounds remained at biologically insignificant levels. Descriptive statistics including means and standard deviations were computed for each group. Due to the primarily qualitative nature of GC-MS data and the minimal leaching observed, further inferential statistics were deemed unnecessary.

In conclusion, while aligner materials demonstrate strong chemical stability, especially in short-term usage, prolonged exposure under harsh conditions may lead to trace compound release. However, the compounds identified pose no known clinical risk, supporting the continued safe use of these materials in orthodontic practice. Future research involving retrieved appliances and extended intraoral simulations would further clarify their long-term biocompatibility.

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Annexure

ANNEXURE I

ETHICAL CLEARANCE

SANTOSH
Deemed to be University
(Established u/s 3 of the UGC Act, 1956)

F. No. SU/2023/2480[19] Dated: 26.09.2023

Subject: Grant of Ethical Clearance for Thesis Protocol Topic "Comparative analysis of material leached from different clear aligner system: an in vitro study", submitted by **Dr. AMRIT SINGH,** MDS student of Batch 2022-2023, Department of **ORTHODONTICS & DENTOFACIAL ORTHOPAEDICS**.

*****

With reference to his/her request for grant of Ethical Clearance for the Thesis Protocol entitled "Comparative analysis of material leached from different clear aligner system: an in vitro study", **Dr. AMRIT SINGH** is informed that the Thesis Protocol submitted by him/her was considered by the **Screening Committee** of the Santosh Dental College & Hospital in its meetings held on 07.02.2023 & 08.02.2023. The recommendations of the **Screening Committee** were considered in detail by the Institutional **Ethics Committee** in its meeting held on **23.09.2023** and the same was **approved** by the **Ethics Committee**.

He /She is informed accordingly for further necessary action.

[DR. NARESH SHARMA]
REGISTRAR

Dr. AMRIT SINGH
MDS student of batch 2022-2023
Department of ORTHODONTICS & DENTOFACIAL ORTHOPAEDICS
Email: amrit3198@gmail.com

Copy to:
1. The Vice Chancellor
2. The Dean, SDC&H
3. The Dean – Research
4. The Dean – Academics
5. The Director - IQAC
6. **Dr. Parvinder Kaur** (Guide)
7. HOD of **ORTHODONTICS & DENTOFACIAL ORTHOPAEDICS**

ANNEXURE II

GC-MS REPORT OF TESTS

Injection Details				
No.	Injection Name	Position	Type	Level
1	Ethyl Acetate	1	Unknown	
2	Ethyl Acetate	1	Unknown	
3	Ethyl Acetate	1	Unknown	
4	PET 100	2	Unknown	
5	PET 75	3	Unknown	
6	PET 50	4	Unknown	
7	PET 25	5	Unknown	
8	PET 100	2	Unknown	
9	PET 75	3	Unknown	
10	PET 50	4	Unknown	
11	PET 25	5	Unknown	
12	PET 100	2	Unknown	
13	PET 75	3	Unknown	
14	PET 50	4	Unknown	
15	PET 25	5	Unknown	
16	PE 100	6	Unknown	
17	PE 75	7	Unknown	
18	PE 50	8	Unknown	
19	PE 25	9	Unknown	
20	PE 100	6	Unknown	
21	PE 75	7	Unknown	
22	PE 50	8	Unknown	
23	PE 25	9	Unknown	
24	PE 100	6	Unknown	
25	PE 75	7	Unknown	
26	PE 50	8	Unknown	
27	PE 25	9	Unknown	
28	PE 100	6	Unknown	
29	PC 100	10	Unknown	
30	PC 75	11	Unknown	
31	PC 50	12	Unknown	
32	PC 25	13	Unknown	
33	PC 100	10	Unknown	
34	PC 75	11	Unknown	
35	PC 50	12	Unknown	
36	PC 25	13	Unknown	
37	PC 100	10	Unknown	
38	PC 75	11	Unknown	
39	PC 50	12	Unknown	
40	PC 25	13	Unknown	

ANNEXURE III

GC-MS REPORT OF RESULT

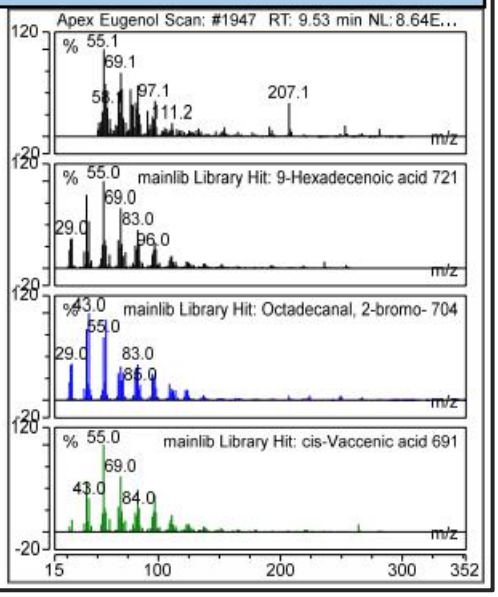
Library Search Results

Ret.Time: 9.527

Hit# 1
Component: 9-Hexadecenoic acid
SI: 721
RSI: 741
Library: mainlib

Hit# 2
Component: Octadecanal, 2-bromo-
SI: 704
RSI: 704
Library: mainlib

Hit# 3
Component: cis-Vaccenic acid
SI: 691
RSI: 712
Library: mainlib



ANNEXURE IV

MASTER CHART - 1

7 day immersion

Ethanol Concentration (%)	Material	Replicate 1	Replicate 2	Replicate 3
0.25	PE	ND	ND	ND
0.25	PET-G	ND	ND	ND
0.25	PCTC	ND	ND	ND
0.5	PE	ND	ND	ND
0.5	PET-G	ND	ND	ND
0.5	PCTC	ND	ND	ND
0.75	PE	0.500	0.500	0.502
0.75	PET-G	0.501	0.514	0.502
0.75	PCTC	0.501	0.502	0.500
1	PE	0.500	0.500	0.501
1	PET-G	0.511	0.507	0.512
1	PCTC	0.512	0.509	0.507

ANNEXURE V

MASTER CHART - 2

14 days immersion

Ethanol Concentration (%)	Material	Peak	RT	Replicate 1	Replicate 2	Replicate 3
0.25	PE	Peak 1		0	0	0
0.25	PE	Peak 2		0	0	0
0.25	PET-G	Peak 1		0	0	0
0.25	PET-G	Peak 2		0	0	0
0.25	PCTC	Peak 1		0	0	0
0.25	PCTC	Peak 2		0	0	0
0.5	PE	Peak 1	15.643	1.0900	1.005	1.060
0.5	PE	Peak 2		0	0	0
0.5	PET-G	Peak 1	15.637	3.051	3.120	3.187
0.5	PET-G	Peak 2	17.588	1.386	1.414	1.359
0.5	PCTC	Peak 1	15.640	1.214	1.344	1.236
0.5	PCTC	Peak 2	17.558	0.880	0.923	0.934
0.75	PE	Peak 1	15.633	2.969	2.781	2.831
0.75	PE	Peak 2	17.555	1.176	1.211	1.106
0.75	PET-G	Peak 1	15.630	7.391	7.460	7.550
0.75	PET-G	Peak 2	17.552	2.156	2.190	2.244
0.75	PCTC	Peak 1	15.837	3.136	3.210	3.264
0.75	PCTC	Peak 2	17.555	1.668	1.754	1.632
1	PE	Peak 1	15.626	9.203	9.111	9.257
1	PE	Peak 2	17.548	2.74	2.930	2.850
1	PET-G	Peak 1	15.620	13.720	13.550	13.480
1	PET-G	Peak 2	17.541	4.860	4.821	4.920
1	PCTC	Peak 1	15.623	5.096	5.250	5.290
1	PCTC	Peak 2	17.548	2.460	2.520	2.430

ANNEXURE VI

TESTING CERTIFICATE

औषधि मानकीकरण अनुसंधान संस्थान
DRUG STANDARDIZATION RESEARCH INSTITUTE
[केंद्रीय यूनानी चिकित्सा अनुसंधान परिषद]
(Central Council for Research in Unani Medicine)
Ministry of Ayush, Govt. of India
Accredited by NABL for ISO/IEC 17025:2017

Dated: 21/05/2025

Analytical Testing Certification

This is to certify that the analytical tests for the thesis study "Comparative analysis of compounds leached from different clear aligner materials: An in-vitro study" were conducted at the Drug Standardization Research Institute (DSRI), Ghaziabad. The necessary laboratory support and instrumentation were provided by our facility for the completion of the experimental work. We confirm that the tests were carried out as per standard procedures. We also extend our best wishes to Dr. Amrit for the successful submission of the thesis.

Dr. Asma Sattar Khan
Research Officer In-charge

डा. अस्मा सत्तार खाँन
Dr. ASMA SATTAR KHAN, M.Sc.Ph.D
अनुसंधान अधिकारी प्रभारी / Research Officer In-Charge
औषधि मानकीकरण अनुसंधान संस्थान, गाजियाबाद
Drug Standardization Research Institute, Ghaziabad

पी. सी. आई. एम. एवं एच. परिसर, द्वितीय तल, कमला नेहरु नगर, गाजियाबाद – 201002 (उत्तर प्रदेश)
PCIM&H Campus, II[nd] Floor, Kamla Nehru Nagar, Ghaziabad – 201002 (Uttar Pradesh)
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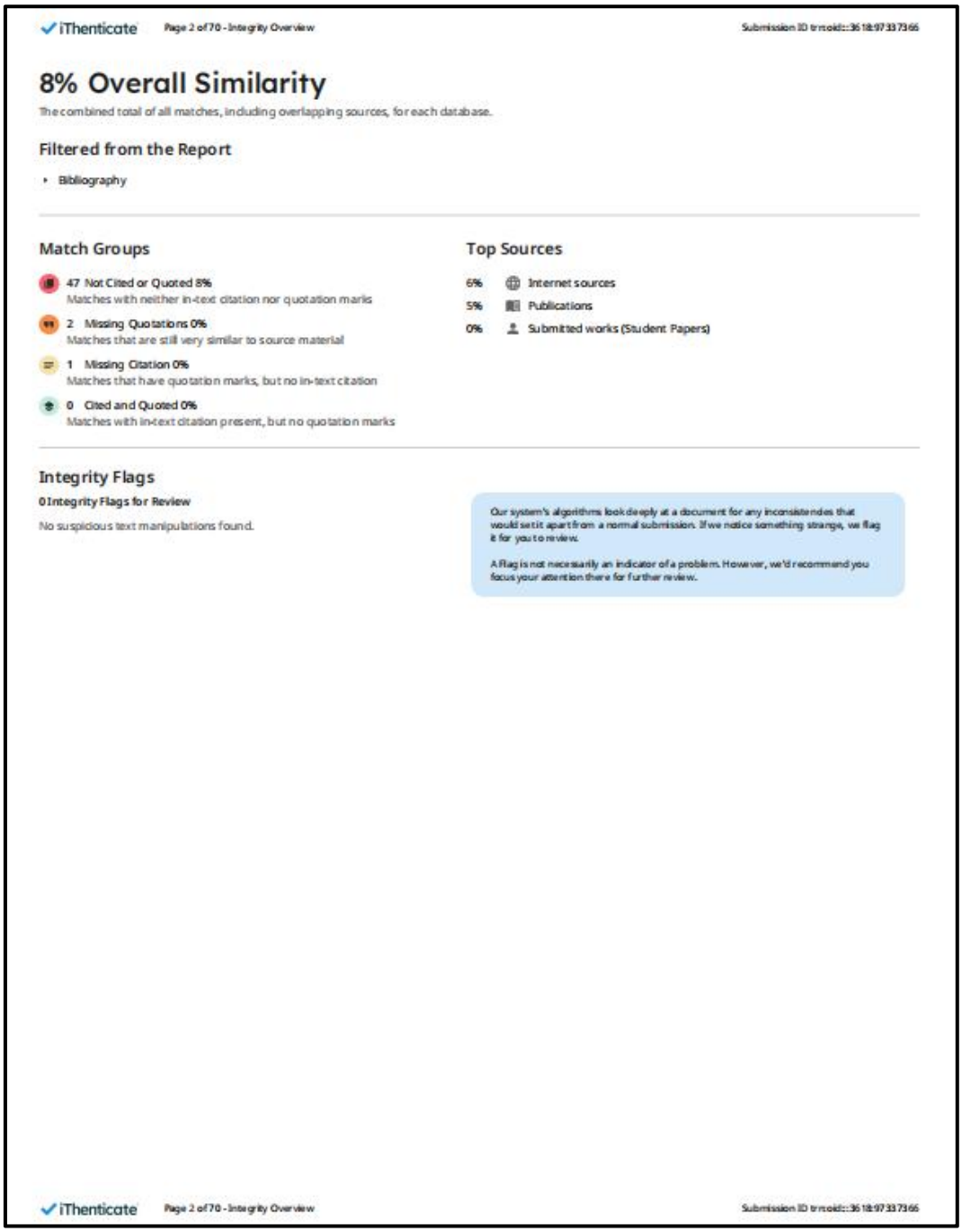
ANNEXURE VII

ANTI-PLAGIARISM CHECK REPORT



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0 Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

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